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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07C 49/84, A61K 31/12

A1

(11) International Publication Number: **WO 95/06628**

(43) International Publication Date: 9 March 1995 (09.03.95)

(21) International Application Number: PCT/DK94/00332

(22) International Filing Date: 2 September 1994 (02.09.94)

(30) Priority Data:
0999/93 3 September 1993 (03.09.93) DK

(71) Applicant *(for all designated States except US)*: STATENS SERUMINSTITUT [DK/DK]; Artillerivej 5, DK-2300 Copenhagen S (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KHARAZMI, Arsalan [DK/DK]; Gudrunsvej 17, DK-2920 Charlottenlund (DK). CHRISTENSEN, Søren, Brøgger [DK/DK]; Åtoften 187, DK-2990 Nivå (DK). MING, Chen [CN/DK]; Henrik Harpestrengs Vej 5, 1.th., DK-2100 Copenhagen Ø (DK). THEANDER, Thor, Grundtvig [SE/DK]; Vejlesøvej 46, DK-2840 Holte (DK).

(74) Agent: PLOUGMANN & VINGTOFT A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).

(81) Designated States: AM, AT (Utility model), AU, BB, BG, BR, BY, CA, CN, CZ, CZ (Utility model), DE (Utility model), DK (Utility model), EE, FI, FI (Utility model), GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, SK (Utility model), TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TREATMENT AND PROPHYLAXIS OF DISEASES CAUSED BY PARASITES OR BACTERIA

(57) Abstract

Aromatic compounds, or prodrugs thereof, which contain an alkylating site and which are capable of alkylating the thiol group in N-acetyl-L-cysteine, in particular bis-aromatic α,β-unsaturated ketones, are used for the preparation of pharmaceutical compositions or medicated feed, food or drinking water for the treatment or prophylaxis of diseases caused by microorganisms or parasites, in particular protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria*, intestinal protozoa such as *Trichomonas* and *Ciardia*; *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Capillaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodacthylogurus, Acantocephalus, Ichthylophtherius, Botrecephalus*; and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria* and *Salmonella*. Preferred compounds have the formula (II): X_m-Ph-C(O)-CH=CH-Ph-Y_n, wherein each phenyl group (Ph) may be mono- or polysubstituted; X and Y designate AR_H or AZ, wherein A is O, S, NH or N(C_{1-6} alkyl), R_H designates aliphatic hydrocarbyl, and Z is H or a masking group which is decomposed to liberate AH; m is 0, 1 or 2, and n is 0, 1, 2 or 3, whereby, when m is 2, then the two X are the same or different, and when n is 2 or 3, then the two or three Y are the same or different, with the proviso that n and m are not both 0. As examples of such compounds, chalcones, e.g. licochalcone A (obtainable i.a. from batches of Chinese licorice root of Glycyrrhiza species, e.g. *G. uralensis* or *G. inflata*) as well as hydroxy, alk(en)yl and/or alk(en)yloxy analogues thereof are active *in vitro* and/or *in vivo* against i.a. *L. major* and *P. falciparum*.

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TREATMENT AND PROPHYLAXIS OF DISEASES CAUSED BY PARASITES OR BACTERIA

The present invention relates to the use of a particular class of aromatic compounds, in particular bis-aromatic α,β-unsaturated ketones, most of which are novel compounds, for the treatment or prophylaxis of a number of serious conditions caused by microorganisms or parasites, in particular protozoa such as *Leishmania*, *Plasmodia*, and *Coccidia* such as *Eimeria*, and intracellular bacteria, including *Legionella* and *Mycobacteria*. The invention also relates to the novel bis-aromatic α,β-unsaturated ketones and methods of preparing them, as well as to pharmaceutical and antiparasitic compositions.

Parasitic diseases, among these malaria and leishmaniasis, are, on a world basis, among the most important diseases. Most of the effective drugs against the diseases have many side effects for which reason it is not possible to maintain the treatment or prophylaxis of specific diseases for years.

Recently, the development of resistance against the available drugs against particularly malaria and leishmania parasites has been reported.

Especially malaria and leishmaniasis remain serious diseases despite the efforts to control the diseases and reduce their prevalence by vector eradication and drug treatment.

Various species of the protozoan parasite *Leishmania* cause a broad spectrum of diseases ranging from the cutaneous healing skin lesions caused by *L. major* to a fatal visceral form of the disease called kala azar caused by *L. donovani* (Manson-Bahr, 1987). Leishmaniasis are widespread in many parts of the world with highest prevalence in Africa, Asia, and Latin America (WHO, 1989). Recently an increasing number of AIDS patients are becoming infected with *Leishmania* (Brenguer, 1989; Flegg 1990).

Therapy of patients with leishmaniasis still poses a serious problem. Most of the available antileishmanial drugs exhibit considerable toxicity and there are reports of large scale clinical resistance to the conventional antimonial drugs. No effective, safe, and nontoxic antileishmanial drug is available at present. There are also reports of large scale clinical drug resistance in visceral leishmaniasis. (TDR News No. 34, 1990)

Malaria, another parasitic disease, is also a serious health problem. Human malaria is caused by four species of the protozoan genus, *Plasmodium*. The species *Plasmodium falciparum* is the most dangerous, causing acute severe infections that are often fatal,

especially in young children and immigrants entering endemic areas.

Each year, several hundreds of millions of human beings are affected by the parasitic disease malaria. The treatment and prophylaxis of malaria has been difficult because the available drugs exhibit severe side effects, and furthermore, the *Plasmodia* are showing increasing resistance towards the drugs (Ann (WHO) 1990). Today, in some areas of the world such as Thailand, multiple drug resistance is so prevalent that there is little to choose from in the terms of effective prophylaxis or therapy (Schulster & Milhous, 1993).

Coccidial protozoa such as *Eimeria tenella* are some of the most important parasites causing disease in poultry resulting in significant economic loss. There are problems with resistance development against some of the available anticoccidial drugs used in prophylaxis and treatment of these diseases, for which reason there is a need for development of new anticoccidial drugs.

Also, *Babesia* species cause devastating damage to cattle in many parts of the world, and there is a need for the development of safe, effective and inexpensive drugs to control these diseases.

Thus, there is a great need for effective drugs against parasitic diseases, especially for drugs exhibiting none or only less severe side effects.

According to the present invention, it has been found that a class of aromatic compounds, said class comprising compounds containing an alkylating site, show a remarkable capability of effectively suppressing the growth of parasitic protozoa and intracellular bacteria, which compounds at the same time can be so chosen that they are tolerable to animal cells such as human cells. This valuable selective activity of such alkylating aromatic compounds seems to be based on their capability of interfering with oxygen metabolism in the parasites by destroying their mitochondria, at concentrations at which the compounds, while thus being harmful to the microorganisms, do not affect the mitochondria of the animal cells.

Without being limited to any particular theory, it is believed that the capability of the compounds to alkylate nucleophilic groups in biomolecules, as evidenced by their capability of alkylating the thiol group of N-acetyl-L-cysteine, is of importance for the antimicrobial effect.

In accordance with this, the present invention, in its broadest aspect, relates to the use of an aromatic compound which contains an alkylating site, and which is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the prepara-

tion of a pharmaceutical composition or a medicated feed, food or drinking water for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

The aromatic compound may in many cases advantageously be used in the form of a prodrug of the aromatic compound, and it will be understood that the present broadest aspect of the invention encompasses the use of such prodrugs.

From the description which follows, it will be seen that a large number of aromatic compounds which show the above-mentioned selective effect are compounds which have one or several electron-donating groups such as hydroxy or derivatives thereof substituted on an aromatic ring. It is believed that the above-described selectivity is obtained through such adequate substitution which modifies the alkylating potency.

As appears from the following, convenient and reproducible *in vitro* tests have been devised to test the selectivity of aromatic N-acetyl-L-cysteine-thiol-alkylating compounds, and based on a large number of tested compounds, it has been found that the above-mentioned aromatic N-acetyl-L-cysteine-thiol-alkylating compounds in which one or several electron-donating groups such as hydroxy or derivatives thereof is/are present on an aromatic ring, are almost consistently capable of showing a useful selectivity, resulting in effective suppression of the growth of pathogenic microorganisms or parasites in concentrations which are well tolerated by animal cells.

The *in vitro* tests involve establishing the inhibition of the multiplication of the protozoa or bacteria on the one hand and the animal cells on the other hand by determining the inhibition of the uptake of radiolabelled precursors as an indication of the inhibition of the growth of the parasite or the animal cells in the presence of the test compound in the concentration in question (see Example 4 herein).

The tests involve a particularly suitable assay for assessing the tolerability of the aromatic alkylating compounds to animal cells, that is, an assay based on the assessment of the reduction caused by the compound on the thymidine uptake by lymphocytes of the animal in the Lymphocyte Proliferation Assay (LPA) which is the assay described in greater detail in Example 4.

It has also been found that compounds which are found to be promising in the *in vitro* model also cure animals infected with leishmania and malaria parasites, respectively, such as was shown in a suitable model involving intraperitoneal administration of the compounds to mice or hamsters (see Examples 5, 6 and 7).

Furthermore, it has been found that compounds with antileishmanial and antimalarial activity exhibit inhibitory effect on the growth of intracellular bacteria such as *Mycobacteria* which causes tuberculosis in humans, and *Legionella* which causes legionnaires disease in humans (see Examples 8 and 9).

The fact that these compounds exhibit strong antiparasitic activity against several species of two important human protozoan parasites, *Plasmodium* and *Leishmania*, and against *Eimeria tenella*, the most important parasite in poultry (see Example 10) makes it justified to presume that these compounds will also be strongly active against important veterinarian protozoan parasites such as *Babesia* in cattle, which is intraerythrocytic similar to the malaria parasite, other *Coccidia* in poultry, and *Pseudodactylogurus* or *Trichodina* in fish.

Furthermore, based on the broad spectrum antimicrobial activity of the compounds (see Examples 8 and 9), it can be assumed that these compounds have similar activity against other microorganisms such as *Salmonella*, and *Trichinella*, and quite generally against a broad range of microorganisms as defined herein, in particular aerobic microorganisms and, among those, in particular microorganisms which are found in tissues and host cells of an infected animal.

While it has been established that the alkylating site may be a carbon-carbon double bond conjugated with a carbonyl group, it is contemplated, based on general chemical considerations, that it may also be a carbon-carbon triple bond conjugated with a carbonyl group, or an epoxy group. The carbonyl group may be the carbonyl group of an aldehyde or a ketone, or it may be the C=O group of a carboxylic acid group or a derivative thereof such as an ester.

In a preferred class of compounds, the carbonyl group is a ketonic carbonyl group which is further conjugated with an aromatic ring, such as a phenyl group. In this case, the phenyl group may carry electron-donating groups, confer what is discussed

above, in particular one or several hydroxy groups or derivatives thereof. In the case of hydroxy groups, these may be masked in order to prevent metabolism, confer the detailed discussion further below. The masking groups are preferably chosen from groups from which the free phenol may be released in the body, either enzymatically or non-enzymatically.

Considering that human lymphocytes are representatives of sensitive animal cells, it is, as a general rule, preferred according to the present invention that the aromatic alkylating compound is one which, in a concentration in which it causes less than 50% reduction, preferably less than 40% reduction, and more preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using phytohemagglutinin (PHA), meets at least one of the criteria a)-d) defined in claim 2.

With reference to the *in vitro*, and in particular the *in vivo* tests described herein, interesting and important aspects of the invention are the treatment and prophylaxis of diseases by *Leishmania, Plasmodium, Coccidia,* including *Eimeria, Mycobacteria* and *Legionella,* such as defined in claims 4-13 herein.

It is preferred that the compounds to be used according to the invention are compounds which meet all of the criteria a) to d), because this is an indication of a broad-spectred activity and selectivity.

Thus, particular important diseases to be treated or prevented by means of the composition prepared according to the invention are human leishmaniasis caused by *Leishmania donovani, L. infantum, L. aethiopica, L. major, L. tropica, L. mexicana complex,* or *L. braziliensis complex* or human malaria caused by *Plasmodium falciparum, P. ovale, P. vivax,* or *P. malariae* as well as parasitic diseases in livestock, such as *Babesia* in cattle, or a parasitic disease in birds, such as a disease caused by *Coccidia* such as *Eimeria tenella* in poultry such as chicken or turkey, or a parasitic disease in fish, such as *Pseudodactylogurus* or *Trichodina.*

The aromatic compound is preferably one which contains an aromatic ring attached to the alkylating site. As indicated above, the compound in particular one which has electron-donating groups attached to an aromatic ring.

In the aromatic compound, the alkylating site is typically a double or triple bond conjugated with a carbonyl group which carbonyl group optionally is further conjugated with an aromatic ring such as a phenyl group, the aromatic ring attached to the alkylating site preferably containing at least one electron-donating group such as an oxygen, nitrogen or sulphur function such as hydroxy, alkoxy (e.g. methoxy),

amino, alkylamino, dialkylamino, mercapto, or alkylthio. It is preferred that the electron-donating group(s) is/are attached to the aromatic ring in a position next to and/or most remote relative to the position through which the aromatic ring is attached to the alkylating site.

The important human malaria parasites with which hundreds of millions of humans are infected, are *Plasmodium falciparum, P. ovale, P. vivax,* and *P. malariae*. In particular, *Plasmodium falciparum* is the most important human parasite and the number one parasite killer of mankind. The malaria parasites show widespread resistance against almost all available antimalarial drugs. For this reason, the fact that a new class of antimalarial drugs, chemically unrelated to the known antimalarial drugs has been provided, is a feature of the invention which is of great importance. Another important aspect of the invention is that malaria parasites resistant against Chloroquine, the most commonly used antimalarial drug, show very high degree susceptibility to the compounds described herein. Malaria disease will not be brought under control without drugs which are effective against the drug resistant strains of the parasite.

Another important aspect of the invention is the antileishmanial activity of the compounds defined above. Visceral leishmaniasis, caused by *Leishmania donovani* or *L. infantum,* inflicts several million people in the world, and this disease recently appears to be a major problem for AIDS patients coming in contact with Leishmania parasites, combined with large scale clinical resistance in endemic areas such as India (which is announced "alarming" by the World Health Organization). Other major diseases are diseases caused by other species of Leishmania, such as *L. aethiopica, L. major, L. tropica, L. mexicana complex,* and *L. braziliensis complex*. Some of these species cause severe disfiguring and morbidity in millions of humans in Central and South America and many parts of Africa.

In one preferred aspect, the invention relates to the use of an aromatic compound which is a bis-aromatic α,β-unsaturated ketone of the general formula I as defined in claim 15 or 16.

In particular, the bis-aromatic α,β-unsaturated ketones act by selectively destroying the cells of the microorganisms or cells of multicellular parasites; as will appear from the below discussion and the examples herein, the bis-aromatic α,β-unsaturated ketones in appropriate concentration ranges will selectively kill the microorganisms or the multicellular parasites by destroying the cells of the microorganisms or cells of the multicellular parasites while showing a high degree of tolerance for the host cells which are subjected to exposure to the compounds.

As indicated above, it is contemplated (as described in detail in the following description of mechanism) that the mechanism of action is via interference of the O_2-metabolism of the microorganism or parasite in question in that the bis-aromatic α,β-unsaturated ketone inhibits or interferes with the O_2-metabolism of the mitochondria (where applicable) of the microorganism such as the parasite or the O_2-metabolism of the bacteria itself. At the same time, the mitochondria of humans have been found to be able to tolerate the compounds in question in the same concentrations which will inhibit or kill the microorganism or the multicellular parasite. It is this remarkable selectivity of certain classes of bis-aromatic α,β-unsaturated ketones which constitutes the basis of this aspect of the present invention.

Many of the bis-aromatic α,β-unsaturated ketones of the general formula I are novel, and the invention also relates to all such novel bis-aromatic α,β-unsaturated ketones. In claims 35-47, some preferred classes of the novel bis-aromatic α,β-unsaturated ketones are defined, and interesting individual compounds among these are mentioned specifically.

Because the bis-aromatic α,β-unsaturated ketones used according to the invention have been found to be well tolerated by animal cells, including human cells, such as will be explained in detail in the following, and because these properties are contemplated to be possessed by the broader range of aromatic compounds defined above, the invention opens up the possibility of controlling parasitic diseases not only by administration to the animals, including humans, as therapy or prophylaxis, but also by killing the parasite in its vector by spraying or otherwise applying an aromatic compound of the type defined above, such as a bis-aromatic α,β-unsaturated ketone, in the infected areas so that the vector will take up the compound, whereby the parasite will be subjected to the compound. Thus, one aspect of the invention relates to a method for controlling transmission of parasitic diseases caused by parasites which have part of their life cycles in a vector, comprising applying an aromatic compound as defined above, such as a bis-aromatic α,β-ketone of the general formula I, to a locus which is a habitat of the vector so as to eradicate the parasites. The parasites will, in this case, in particular be *Leishmania*, *Plasmodium*, or *Trypanosoma*, and the eradication of the parasite will, depending on the vector's tolerance to the compound, take place with or without concomitant eradication of the vector.

When W in the general formula I is -CR=CR-, it may be either cis or trans configured. It is preferred that it is trans configured. It is often preferred that both groups R are hydrogen, but it is contemplated that also bis-aromatic α,β-unsaturated ketones in which one of or both groups R is/are e.g. methyl or ethyl are of great value with respect to the relevant activity and selectivity/tolerability.

The aromate is preferably phenyl such as illustrated of the examples herein, but it is reasonable to contemplate that any of the aromate types mentioned in claim 15 can be the Ar^1 or Ar^2 of the bis-aromatic α,β-unsaturated ketone, considering that such aromatic rings will affect the electron density in the unsaturated ketone similarly to the two phenyl rings, and that such aromates will also give possibilities for charge transfer complexes and lipophilic interactions with the target molecule, such as do the two phenyl rings. Interesting compounds are defined in greater detail in claim 17.

Apart from the important substitution with X and/or Y as defined in connection with formula I, the aromate may carry other substituents which either will not to any substantial extent detract from the useful effect and selectivity of the bis-aromatic α,β-unsaturated ketones, or will enhance these properties or relevant properties related to the use and utility of the bis-aromatic α,β-unsaturated ketones, e.g., their solubility (such as when the bis-aromatic α,β-unsaturated ketones carry a nitrogen-containing basic group or a carboxyl group which can form water-soluble salts with pharmaceutically acceptable counter ions).

Among the bis-aromatic α,β-unsaturated ketones of the general formula I, the preferred ones are generally those in which A is O, mainly because of their excellent properties with respect to activity and selectivity/tolerability, such as will appear from the results reported herein. However, it is well known that the oxygen atom in the form of oxy in many biologically active compounds may, with greater or lesser retention of, and indeed in certain cases with enhancement of, the biological activity, be replaced with bioisosteric groups, such as -S-, -NH-, and -N(C_{1-6} alkyl)- as mentioned above.

Based upon the above-mentioned general preference for substituents X and Y which contain -O- (but taking into consideration that the oxygen atom could be replaced with the a bioisosteric group), this substituent could be called "an oxy-functional substituent". While it is presumed that the activity of the oxy-functional substituent is related to the substituent in the "free" form, that is, to hydroxy when A is -O-, to thiolo when A is -S-, and to amino or monoalkylamino when A is -NH- or -N(C_{1-6}-alkyl)-, very interesting results obtained with bis-aromatic α,β-unsaturated ketones of the formula I where X or Y is alkenyloxy raise the intriguing question whether the active form in theses cases is the alkenyloxy-substituted form, or whether the alkenyloxy group is converted to a hydroxy group , maybe even by the microorganism or parasite itself, before the bis-aromatic α,β-unsaturated ketone exerts it action. As will be understood, this possibility is covered by the definition R_H above, while the definition of Z , when Z is not hydrogen, is adapted to represent "prodrug" forms which, in accordance with well known principles used in the construction of suitable administration embodiments of chemical compounds containing, e.g., free hydro groups as substituents on aromatic rings, will be decomposed in the animal body to

result in the corresponding compound in which Z is hydrogen.

In a preferred embodiment, the bis-aromatic α,β-unsaturated ketone has the general formula II as defined in claim 18 and 19.

In especially preferred embodiments, the substituent or substituents on the phenyl groups is/are selected from methyl, ethyl, propyl, isopropyl, tert.-butyl, prop-2-enyl, 1,1-dimethylpropyl, 1,1-dimethylprop-2-enyl, 3-methylbutyl, and 3-methylbut-2-enyl.

Particularly interesting classes and subclasses of the bis-aromatic α,β-unsaturated ketones are defined in claims 20-34.

The host animals to be treated, either to obtain a therapeutic effect, or to obtain a prophylaxis or protection against infection, are primarily vertebrates such as birds, fish and mammals, including humans. It is evident that with respect to some of the microorganisms and multicellular parasites mentioned above, the host to be treated is defined once the microorganism or multicellular parasite is given. Thus, for example, when the microorganism is *Leishmania*, the hosts to be treated are humans or dogs; when the microorganism is *Theileria*, the animals to be treated are cattle, sheep and goats; when the microorganism is *Eimeria*, the animals to be treated are chickens and turkeys.

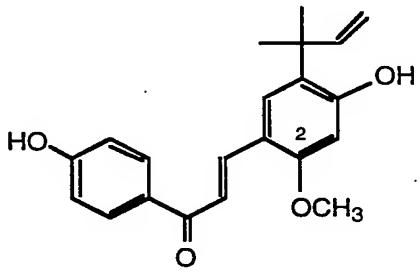
Based upon findings as explained in the examples below, it is presumed that the mechanism of action of the bis-aromatic α,β-unsaturated ketones of the general formulae I and II above, and prodrugs thereof, is as follows:

The bis-aromatic α,β-unsaturated ketones severely damage the mitochondria of the parasites. Mitochondria are oval-shaped organelles, typically about 2 μm in length and 0.5 μm in diameter, located intracellulary in all organisms except bacteria. Mitochondria have two membrane systems, an outer membrane and an extensive, highly folded inner membrane, hence there are two compartments in mitochondria: the intermembrane space between the inner membrane and the outer membrane, and the matrix, which is bounded by the inner membrane.

Mitochondria are the organelles involved in the O_2-metabolism of the cell. Oxidative phosphorylation is the process in which ATP is formed as electrons are transferred from NADH or $FADH_2$ to O_2 by a series of electron carriers. This is the major source of ATP in aerobic organisms. Oxidative phosphorylation is carried out by respiratory assemblies located as an integral part of the inner mitochondrial membrane. The outer membrane is quite permeable to most small molecules and ions.

From B. Inoue et al, *J. Toxicol. Sci.* 7 (1982), 245-254, it is known that echinatin, 4-hydroxychalcone, chalcone, and 3,4'-dihydroxychalcone cause deterioration of respiratory control and oxidative phosphorylation of isolated rat liver mitochondria. The present inventors have found that bis-aromatic α,β-unsaturated ketones and derivatives thereof of the general formula I or II cause deterioration of respiratory control and oxidative phosphorylation of mitochondria of parasites in concentrations that are so small that the mitochondria of the animal cell are not affected.

Due to the interference with the O_2-metabolism of the mitochondria the mitochondria are destroyed and as a consequence the cell to which the mitochondria belong is destroyed. Thus, the compound known as licochalcone A and having the formula



does not appear to exhibit any toxicity in animal cells even at fairly high concentrations, cf. the data given in Example 4 herein. Thus, licochalcone A is an important potential antiparasitic, in particular, antimalarial and antileishmanial drug. However, as appears from the experiments reported in the examples herein, the surprising effect and selectivity found is not limited to licochalcone A, but is characteristic of the class of bis-aromatic α,β-unsaturated ketones discussed herein and, for the reasons given above, is believed to apply more broadly to the aromatic compounds defined above.

bis-Aromatic α,β-unsaturated ketones as defined herein have been found to have effect on the *Leishmania* parasite in the amastigote phase as well as in the promastigote phase. This means that the compounds in question are both useful in the prophylaxis of leishmaniasis, because of the effect against the promastigotes, and in the treatment of the disease, because of the effect against the amastigotes. Again, this is believed to apply more broadly to the aromatic compounds defined above.

It is contemplated that the aromatic compounds, such as the bis-aromatic α,β-unsaturated ketones defined herein interfere with the O_2-metabolism of the cytoplasmic membrane corresponding to the interference with the O_2-metabolism of the mitochondria of higher developed organisms, thereby destroying the bacteria.

As mentioned above, important findings on which the present invention is based are

not only the remarkable efficiency of the bis-aromatic α,β-unsaturated ketones with respect to destroying the pathogenic microorganisms, but also the high degree of selectivity which they show with respect to the pathogenic microorganisms as contrasted to animal cells, including human cells. Thus, as will appear from the data given in the examples below, bis-aromatic α,β-unsaturated ketones have been found to be substantially harmless to human cells in concentrations at which they effectively control the parasites. This selectivity was surprising. Moreover, as appears from the examples, a still much higher activity against the microorganisms is found when the microorganisms are present in tissue, such as in cells, such as will be the case in the actual therapeutic use. In many cases, a further increase by a factor 10 in the selectivity is seen.

Peliminary experiments involving oral administration of licochalcone A to mice and rats and injection of licochalcone A to mice indicate that in animals such as mammals, the bis-aromatic α,β-unsaturated ketones which possess a free phenolic hydroxy group will be eliminated from the blood stream already after the first passage to through the liver. This is in accordance with what is known about the metabolism of other phenolic compounds. For this reason, an interesting aspect of the invention is constituted by compounds in which the phenolic hydroxy group or groups or bioisosteric other group or groups AZ are masked, in other words, the so-called prodrugs, that is, compounds which are readily decomposed under conditions prevailing in the animal body to liberate the free groups which are associated with the active forms of the drugs.

The prodrugs used according to the invention are, e.g., compounds of the general formula I or II in which Z is a group which is readily decomposed under conditions prevailing in the animal body to liberate the group AH. As an important example, when A is O such as is the case in important compounds used according to the invention, it is preferred that Z is a group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH.

The establishment of prodrug forms suitable in connection with particular substituents in drugs is based upon the fact that certain types of groups will tend to be decomposed in the animal body in accordance with various decomposition pathways. Thus, among the specific prodrug groups (A)-(E) (see claim 16), the groups (A), (D), and (E) are groups which will be decomposed by esterases to result in the corresponding free group such as the hydroxy group. The group (B) will be subjected to removal of one of the methyl groups in the liver, and the group thus formed will be relatively readily decomposable in plasma. The oxy-containing groups (C) are groups which are relatively labile under acidic conditions and, as thus, are adapted to be decomposed, e.g., under the conditions under which *Leishmania* amastigotes exist in the human body,

that is, in macrophages. Quite generally, the prodrug group Z will be one which prevents the active molecule from being converted, in the liver, to a form which, from a practical point of view, will be inactive and quickly will be eliminated from the animal body, such as the forms where free phenolic OH groups are sulphated in the liver or are coupled to glucuronic acid in the liver.

In preferred prodrug embodiments, Z is a group selected from the groups (A)-(E) as defined above. Examples of particularly preferred groups Z are pivaloyl, pivaloyloxymethyl and N,N-dimethylcarbamoyl.

The above considerations concerning prodrug derivatives of hydroxy groups in the compounds of the general formula I or II also apply to other hydroxy group-containing aromatic alkylating compounds as defined above.

Formulation of pharmaceutical compositions

The administration route of the aromatic compound as defined above, such as the bis-aromatic α,β-unsaturated ketones of the general formula I, may be of any suitable route which leads to a concentration in the blood corresponding to a therapeutic concentration by the oral route, the parenteral route, the cutaneous route, the nasal route, the rectal route, the vaginal route and the ocular route. It should be clear to a person skilled in the art that the administration route is dependant on the compound in question, particularly, the choice of administration route depends on the physico-chemical properties of the compound together with the age and weight of the patient and on the particular disease and the severity of the same.

The aromatic compounds as defined above, such as the bis-aromatic α,β-unsaturated ketones or derivatives thereof, may be contained in any appropriate amount in a pharmaceutical composition, and are generally contained in an amount of about 1-95% by weight of the total weight of the composition. The composition may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, aerosols and in other suitable form. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology".

Pharmaceutical compositions according to the present invention may be formulated to release the active compound substantially immediately upon administration or at any substantially predetermined time or time period after administration. The latter type of compositions are generally known as controlled release formulations.

Controlled release formulations may also be denoted "sustained release", "prolonged release", "programmed release", "time release", "rate-controlled" and/or "targeted release" formulations.

In general, two basically different strategies can be applied in order to obtain a controlled release formulation in which the rate of release outweighs the rate of metabolism of the compound in question. In the first strategy, the principle aims at changing the properties of the active drug substance by converting the substance into a masked form. The compounds of the above formulae in which Z is one of the groups (A)-(E) are representatives of this strategy. In the second strategy, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g. various types of controlled release compositions and coatings (formulation-method).

It will be appreciated that a combination of the above-mentioned two methods can be used in controlled release compositions, comprising the aromatic compounds defined above, such as bis-aromatic α,β-unsaturated ketones, according to the invention, e.g., by using a prodrug of the compound in question and then formulating according to the principles mentioned above.

In the present context every pharmaceutical composition is an actual drug delivery system, since upon administration it presents the active drug substance to the body of the organism.

Dosages

The bis-aromatic α,β-ketones are preferably administered in an amount of about 0.1-30 mg per kg body weight per day, such as about 0.5-15 mg per kg body weight per day. The compound in question may be administered orally in the form of tablets, capsules, elixirs or syrups, or rectally in the form of suppositories. Parenteral administration of the aromatic compound defined above, such as the bis-aromatic α,β-unsaturated ketone, is suitably performed in the form of saline solutions of the ketones (or salts thereof) or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, an acid addition salt of a basic compound of the formula I (that is, a compound of the formula I in which either an aromatic ring or a substituent contains a basic nitrogen atom) can be used, or a solubilizer such as ethanol can be applied.

Oral administration. For compositions adapted for oral administration for systemic use, the dosage is normally 1 mg to 1 g per dose administered 1-4 times daily for 1 week to 12 months depending on the disease to be treated.

The dosage for oral administration for the treatment of parasitic diseases is normally 1 mg to 1 g per dose administered 1-2 times daily for 1-4 weeks, in particular the treatment of malaria is to be continued for 1-2 weeks whereas the treatment of leishmaniasis will normally be carried out for 3-4 weeks.

The dosage for oral administration for the treatment of bacterial diseases is normally 1 mg to 1 g per dose administered 1-4 times daily for 1 week to 12 months; in particular, the treatment of tuberculosis will normally be carried out for 6-12 months.

The dosage for oral administration of the composition in order to prevent diseases, in particular, parasitic diseases, is normally 1 mg to 75 mg per kg body weight per day. The dosage may be administered once or twice daily for a period starting 1 week before the exposure to the disease until 4 weeks after the exposure.

Rectal administration. For compositions adapted for rectal use for preventing diseases, a somewhat higher amount of aromatic compounds, such as bis-aromatic α,β-unsaturated ketones or derivatives thereof is usually preferred, i.e. from approximately 1 mg to 100 mg per kg body weight per day.

Parenteral administration. For parenteral administration a dose of about 0.1 mg to about 50 mg per kg body weight per day is convenient. For intravenous administration a dose of about 0.1 mg to about 20 mg per kg body weight per day administered for 1 day to 3 months is convenient. For intraarticular administration a dose of about 0.1 mg to about 20 mg per kg body weight per day is usually preferable. For parenteral administration in general, a solution in an aqueous medium of 0.5-2% or more of the active ingredients may be employed.

Percutaneous administration. For topical administration on the skin a dose of about 1 mg to about 5 g administered 1-10 times daily for 1 week to 12 months is usually preferable.

Transmission control. As mentioned above, the use of the aromatic compounds defined above, such as the bis-aromatic α,β-unsaturated ketones, or derivatives thereof in controlling parasites in their vectors is an interesting and promising aspect of the present invention. The principle is to destroy the parasites in their vectors, thereby preventing transmission of the disease. The data presented herein demonstrate clearly that the promastigote stage, the same form of the parasite which is present in the sandfly vector, of the *L. major* and *L. donovani* parasite, is killed by the bis-aromatic α,β-unsaturated ketones or derivatives thereof. For example, spraying endemic areas for malaria or leishmania or other protozoal diseases transmitted by

their respective vectors will be of an attractive means of controlling such important parasitic diseases.

For the compounds mentioned above containing a double bond, the corresponding compounds in which the bond is a triple bond such as discussed in connection with the general formula I are also very interesting and should be considered correspondingly disclosed herein in connection with each and every structural formula shown herein and each and every compound named herein.

In many cases, it will be preferred to administer the compound defined herein together with another antiparasitic, antimycotic or antibiotic drug, thereby reducing the risk of development of resistance against the conventional drugs, and reducing the amount of each of the drugs to be administered, thus reducing the risk of side effects caused by the conventional drugs. Important aspects of this is the use of the compound against *Leishmania*, where the compound I is combined with another antileishmanial drug, or the antimalarial use of the compound I where the compound I is used together with another antimalarial drug.

As examples of other antileishmanial drugs to be combined with the compounds defined herein may be mentioned pentavalent antimony-sodium gluconate and allopurinol. As examples of other antimalarial drugs to be combined with the compounds defined herein may be mentioned chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine and artemisinin. As an example of an additional antibiotic drug to be combined with the compounds defined herein may be mentioned an antituberculous drug such as isoniazide, ethambutol, pyrazinamide, and rifampicin. As examples of additional antimycotic drugs to be combined with the compounds defined herein may be mentioned amphotericin B, muconarcidol, griseofulvin, and miconazol. As examples of additional antibabesial drugs to be combined with the compounds defined herein may be mentioned quinuronium sulfate, pentamidine isethionate, imidocarb or diminazene. As examples of additional anticoccidial drugs to be combined with the compounds defined herein may be mentioned fulfonamides, amprocid and coccidiostatic agents such as inomycins, in particular monensin and salinomycin.

As examples of additional drugs against fish parasites to be combined with the compounds defined herein may be mentioned benzimidazol and formaldehyde.

One general advantage of the compounds defined herein are their broad-spectered character, which makes it possible to use the compounds as sole medication in cases where the host to be treated is infected with, or suspected to be infected with, more than one of the bacteria and parasites discussed herein, or to use them as supplements

to known antibacterial agents and antiparasitic agents in order to reduce the dose of the conventional antibiotics or antiparasitic agents, thus reducing the risk of side effects, in addition to the above-mentioned advantages with respect to reduction of drug resistance development.

In particular for prophylaxis, the broad-spectered character of the compounds of the general formula I is of great advantage, and may be further augmented by combination with more than one antibacterial or antiparasitic agent, such as combination with both another antileishmanial agent and another antimalarial agent. It is justified to presume that also the other aromatic compounds defined herein will show the same valuable broad-spectered character.

While the above-mentioned compounds of the general formula I are predominantly compounds in which W is -CR=CR-, it should be borne in mind that compounds which correspond to each of the compounds mentioned herein, but in which W is -C≡C-, are also important compounds according to the invention.

As mentioned above, a number of the compounds of the general formula I are known, whereas many of the compounds of the general formula I are novel compounds. The known compounds may be isolated or synthesized in accordance with methods known from the literature or methods analogous thereto. The novel compounds may, likewise, be produced by methods known *per se* or methods which are analogous to such methods. The compounds of the formula I may be pepared by the process claimed in claim 56.

In a process a), compounds of the formula I in which W is -CH=CH- are prepared by reacting a ketone of the general formula I'

$$X\text{-}Ar^1\text{-}CO\text{-}CH_3 \qquad I'$$

with an aldehyde of the general formula I''

$$HCO\text{-}Ar^2\text{-}Y \qquad I''$$

This reaction, which is a condensation reaction, is suitably carried out under acid or base catalyzed conditions. A review of such processes may be found in Nielsen, A.T., Houlihahn, W.J., *Org. React.* **16**, 1968, p 1-444. In particular the method described by Wattanasin, S. and Murphy, S., *Synthesis* (1980) 647 has been found to be very successful. The reaction may suitably be carried out in protic organic solvents, such as lower alcohols (e.g. methanol, ethanol, or tert.butanol), or lower carboxylic acids (formic, glacial acetic, or propionic acid), or in aprotic organic solvents such as ethers

(e.g. tetrahydrofuran, dioxan, or diethyl ether), liquid amides (e.g. dimethylformamide or hexamethylphosphordiamide), dimethylsulfoxide, or hydrocarbons (e.g. toluene or benzene), or mixtures of such solvents. When carrying out the reaction under base catalyzed conditions, the catalyst may be selected from sodium, lithium, potassium, barium, calcium, magnesium, aluminum, ammonium, or quaternary ammonium hydroxides, lower alkoxides (e.g. methoxides, ethoxides, tert.butoxides), carbonates, borates, oxides, hydrides, or amides of lower secondary amines (e.g. diisopropyl amides or methylphenyl amides). Primary aromatic amines such as aniline, free secondary amines such as dimethyl amine, diethyl amine, piperidine, or pyrrolidine as well as basic ion exchange resins may also be used.

Acid catalysts may be selected from hydrogen chloride, hydrogen bromide, hydrogen iodide, sulfuric acid, sulfonic acids (such as paratoluenesulfonic or methanesulfonic acid), lower carboxylic acids (such as formic, acetic or propionic acid), lower halogenated carboxylic acids (such as trifluoroacetic acid), Lewis acids (such as BF_3, $POCl_3$, PCl_5, or $FeCl_3$), or acid ion exchange resins.

A drawback of the base catalyzed condensation is the poor yield obtained if the aromatic ring in which the ketone or the aldehyde or bort is substituted with one or more hydroxy groups. This inconvinience can be overcone by masking the phenolic group by T. Hidetsugu et al. European patent application 0370 461 (1989). Deprotection is easily performed by mineral acids such as hydrochloric acid.

The reaction may be carried out at temperatures in the range of 0-100°C, typically at room temperature. Reaction times may be from 30 min to 24 hours.

In another process b), compounds of the formula I in which W is -C≡C- may be prepared by reacting an activated derivative of a carboxylic acid of the general formula

$$X\text{-}Ar^1\text{-}COOH$$

wherein X and Ar^1 are as defined above, with an ethyne derivative of the formula II'

$$H\text{-}C{\equiv}C\text{-}Ar^2\text{-}Y \qquad II'$$

wherein Ar^2 and Y are as defined above. Reactions of this type are described by Tohda, Y., Sonogashihara, K., Haghara, N., *Synthesis* **1977**, p777-778. It is contemplated that the activated derivative of the carboxylic acid IV may be an activated ester, an anhydride or, preferably, an acid halogenide, in particular the acid chloride. The reaction is normally carried out using the catalysts described by Tohda, Y. *et al.* cited

above, namely copper(I)iodide/triphenylphosphine-palladium dichloride. The reaction is suitably carried out in triethylamine, a mixture of triethylamine and pyridine or triethylamine and toluene under a dry inert atmosphere such as nitrogen or argon. The reaction is generally carried out at reduced temperature such as in the range from -80°C to room temperature, the reaction time typically being from 30 minutes to 6 hours.

In the above reactions, it may be preferred or necessary to protect various sensitive or reactive groups present in the starting compounds of formulas II, III, IV, or V so as to prevent said groups from interfering with the reactions. Such protection may be carried out in a well-known manner, e.g. as described in "Protective Groups in Organic Chemistry" by Theodora Green. For example, in the reaction between the acid derivative IV and the acetylene derivative V, a hydroxy group on Ar^1 and/or Ar^2 may be protected in the form of the methoxymethyl ether, N,N-dimethylcarbamoyl ester, or allyl ether. The protecting group may be removed after the reaction in a manner known *per se*.

An alternative route for the preparation of the compounds of the general formula I goes via the 1,3-dipolar cycloaddition mechanism (Torssell, K.B.G., Weinheim 1988) known as the isoxazoline route.

Thus, by reaction of an aldehyde of the general formula A

$$X\text{-}Ar^1\text{-}CHO \qquad A$$

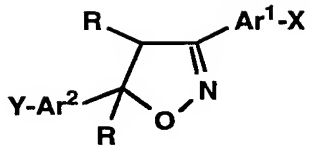
wherein X and Ar^1 are as defined above, with hydroxylamine (e.g. hydroxylaminehydrochloride) using water as a solvent, the corresponding oxime of the general formula B

$$X\text{-}Ar^1\text{-}CH{=}N\text{-}OH \qquad B$$

is formed, and a chlorinating agent (e.g. NCS or t-butylhypochlorite) is added to chlorinate the oxime, which in contact with alkenes of the general formula C

$$CHR{=}CR\text{-}Ar^2\text{-}Y \qquad C$$

in which Ar^2, Y and R are as defined above, will form the corresponding isoxazoline of the formula D



D

The chlorination and the formation of the isoxazoline ring can be performed by a one-pot method. Solvents like methylenechloride, chloroform are most commonly used. By reducing the formed isoxazoline in an aqueous medium, the reduced product will be hydrolyzed to a β-hydroxyketone of the general formula E,

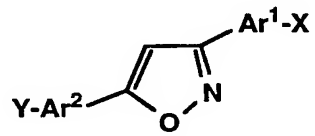
$$X\text{-}Ar^1\text{-}CO\text{-}CRH\text{-}C(OH)R\text{-}Ar^2\text{-}Y \qquad E$$

wherein Ar^1, Ar^2, X and Y are as defined above. This reduction, including the hydrolysis, is a very efficient synthetic tool, and gives products in almost 100% yield. The reduction can be carried out by the use of Ra-Ni together with catalytic amounts of acid or by electrochemical reduction. Optionally, the hydroxy group is substituted with another leaving group such as halide, alkoxy, tosyloxy, or trifluoromethanesulfonoxy, such other leaving group being introduced in a manner known *per se*. When the hydroxy group is not substituted with such other leaving group, this β-hydroxy-ketone (E) is treated with acid (e.g. paratoluenesulfonic acid or a mixture of acetic acid and sodium acetate), whereby water is eliminated, giving the chalcone structure of the general formula (I).

Correspondingly, compounds in which W is -C≡C- can be made via a route where the oxime of the above general formula B is reacted with a halogenating agent and an acetylene of the general formula C1

$$CH{\equiv}C\text{-}Ar^2\text{-}Y \qquad C1$$

wherein Ar^2 is as defined above, is added to form the corresponding isoxazole of the formula D1



D1

wherein Ar^1, Ar^2, X and Y are as defined above, which is then reduced, and the reduction product is hydrolysed to form a β-hydroxyketone of the general formula E1

$$X\text{-}Ar^1\text{-}CO\text{-}CH{=}C(OH)\text{-}Ar^2\text{-}Y \qquad E1$$

wherein Ar^1, Ar^2, X and Y are as defined above, and optionally substituting the hydroxy group with another leaving group such as halide, alkoxy, tosyloxy, or trifluoromethanesulfonoxy, such other leaving group being introduced in a manner known *per se*. By elimination of the leaving group, the compound of the general formula I, wherein W is -C≡C-, is obtained.

Another route for the preparation of the compounds of the general formula I Is the Wittig reaction in which an aldehyde of the general formula F

$$Y\text{-}Ar^2\text{-}CHO \qquad F$$

wherein Ar^2 and Y are as defined above, is treated with a phosphorus ylide (also called a phosphorane) of the general formula G,

$$T_3\text{-}P{=}CH\text{-}CO\text{-}Ar^1\text{-}X \qquad G$$

in which T can be aliphatic, alicyclic or aromatic, to give the chalcone structure of the general formula (I). The Wittig reaction is known as an exceedingly useful method for the synthesis of alkenes.

The aldehyde may be aliphatic, alicyclic or aromatic; it may contain double or triple bonds; it may contain various functional groups, such as OH, OR, NR_2, aromatic nitro or halo, acetal or even ester groups. Double or triple bonds conjugated with the carbonyl also do not interfere; the attack is directed towards the carbonyl carbon atom. The reaction is suitably carried out in aprotic organic solvents such as ethers (e.g. tetrahydrofuran, dioxan, or diethyl ether) or DMSO or mixtures of these. The reaction is normally carried out at temperatures in the range of 0-25°C, but can also be carried out at even lower temperatures.

Phosphoranes of the general formula $Me_nPh_{(3-n)}P{=}CHCOPh$ (Ph = phenyl or substituted phenyl, n = 0,1,2,3) are reported to react with benzaldehyde to give chalcone in good yield (70-90%). According to DE 1.256.642 (1967), the Wittig reaction is used for the preparation of chalcone in 84% yield (Bestmann, H.J., and Kratzer, O.).

Another route for the preparation of compounds of the general formula I is by reacting benzaldehyde with N-α-styrylmorpholine (Birkofer, L., Kim, S.M., and Engels, H.D., Chem. Ber., 95, 1495 (1962)).

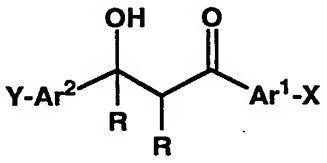
A styrene compound of the general formula H

$$X\text{-}Ar^1\text{-}CV{=}CH_2 \qquad H$$

in which V represents a secondary amino group, is reacted with an aldehyde of the general formula J

$$Y\text{-}Ar^2\text{-}CHO \qquad J$$

to form an intermediate which after hydrolysis and elimination of the secondary amino group gives a compound of the structure K



K

wherein X, Y, Ar^1, Ar^2 and R are as defined above. In the compound K, the hydroxy group may, if desired, be substituted with another leaving group such as alkoxy, tosyloxy, trifluoromethanesulfonoxy or acyloxy in a manner known *per se*. After elimination of HOH or HOTt, wherein Tt is such other leaving group, the chalcone structure of the general structure (I) is obtained.

The usefulness of enamines as intermediates lies in the fact that the β-carbon of the double bond of the enamine has nucleophilic character. This will make the reaction between the enamine and the aldehyde possible.

Another route for the synthesis of compounds of the general formula I is by reacting derivatives of cinnamic acid which is based on the fact that cinnamic acid can react with aromatic compounds (e.g. phenols and benzene).

Cinnamic acids of the general formula L

$$Y\text{-}Ar^2\text{-}CH{=}CH\text{-}COQ \qquad L$$

in which Q is either a hydroxy group, a carboxylate or a halogen, are condensed with aromates of the general formula M

$$X\text{-}Ar^1 \qquad M$$

to give α,β-unsaturated ketones of the general formula (I).

The reaction is best carried out in the presence of BF_3 (Starkov, S. P. et al, *Khim. Tekhnol.*, **20**, 1149 (1977)) or polyphosphoric acid (Reichel, L., and Proksch, G., Justus Liebigs Ann. Chem., 745, 59 (1971)). With the former agent, a high preference for para-acylation is observed.

The reaction is preferably carried out in the presence of $AlCl_3$ as a catalyst (Rasschaert, A. et al, *Bull. Soc. Chim. Belges.*, **75**, 449 (1966).

The two latter methods are examples of the Friedel-Craft acylation. Among the reagents (compounds L) used are not only acyl halides or the acids, but also anhydrides. The reaction can be carried out with only very small amounts of catalyst, often just a trace and sometimes without any catalyst at all. Ferric chloride, iodine, zinc chloride, and iron are the most common catalysts used. Proton acids can be used as catalyst when the reagent (compound L) is in its acid form.

In order to prevent acylation of the solvent used, the reaction is often carried out in a non-aromatic fully saturated solvent or in an aromatic solvent with deactivating groups, which prevents acylation, such as, for example, nitrobenzene. The reaction is carried out at wide varying temperatures depending on the nature of the reacting compounds.

The prodrugs of the general formula I can be made directly by processes as described below, the desired prodrug groups being in place in the relevant reagents in question, or free AZ groups in which Z is hydrogen, in particular hydroxy groups, can be converted to the corresponding groups in which Z is one of the groups (A) to (E) as defined above.

(Acyloxy)alkyl-α-ethers such as those of the general formulas (IIDa, IIEa, IIEb, I'Db, I'Eb, IIIDa, IIIEa) can be prepared by reacting the corresponding phenols of the general formulas (IIa, IIb, IIIa) with the appropriate (acyloxy)alkyl-α-halide.

The reaction is most often carried out using acetone or butanone as solvents. A weak base like potassium carbonate may be added as an acid scavenger.

In the case of pivaloyloxymethyl-α-halides, the halogen should be iodine in order to avoid formation of pivalic esters of phenol (Sloan, K.B., and Koch, S. A. M., *J. Org. Chem.* **48** (1983) 3777-3783.)

Carboxylic esters of the phenols of the general formula I (I'Ab, IIAa, IIIAa) may be

prepared by reacting the corresponding phenols (e.g. IIa, IIb, IIIa) with an activated ester (including the α-halomethylesters), an anhydride or, preferably, an acid halogenide, in particular the acid chloride.

The reaction is performed in an aprotic organic solvent such as lower aliphatic ketones like acetone, butanone, aliphatic ethers like tetrahydrofuran, diethylether, or dioxane or a liquid amine like pyridine. The reaction is carried out in the presence of an acid scavenger such as potassium or sodium carbonate, an tertiary aliphatic amine such as triethylamine, or pyridine.

An especially spectacular modification of the method involves the reaction of the phenol with the appropriate anhydride using 4-dimethylaminopyridine or 4-(1-pyrrolidino)pyridine as catalyst. With these reaction conditions, the reaction gives a very high yield.

N,N-Dimethylcarbamic esters of the phenols of the general formula I (I'Bb, IIIBa) may be prepared by reacting the corresponding phenols of the general formula I (IIa, IIb) with an activated derivative of N,N-dimethylcarbamic acid such as an activated ester or, preferably, an acid halide, in particular the acid chloride.

The reaction is carried out in an aprotic organic solvent such as lower aliphatic ketones like acetone, butanone, aliphatic ethers such as tetrahydrofuran, diethylether, or dioxane, or a liquid amine such as pyridine, or a liquid nitrile such as acetonitrile. In general, the reaction is carried out in the presence of an acid scavenger such as potassium or sodium carbonate, a tertiary aliphatic amine such as triethylamine or pyridine

Alternatively, the N,N-dimethylcarbamoyl esters may be prepared by condensing the carbamoylated phenolic benzaldehydes or phenolic acetophenones with the appropriate acetophenones or benzaldehydes, respectively.

The alkoxymethoxy ethers of the general formula I (I'Cb, IICa, IICb, IIICa) are most conveniently prepared by condensing the appropriate ethers of the phenolic benzaldehydes or the phenolic acetophenones with the appropriate acetophenones or benzaldehydes, respectively. They may, however, be prepared by reacting the phenolic chalcones with the appropriate alkyl-α-alkylhalomethyl halide.

The reaction may be carried out in an aprotic organic solvent like a lower aliphatic ketone, such as acetone or butanone, or an ether, such as tetrahydrofuran, dioxane or dioxolane or a liquid nitrile such as acetonitrile. The reaction may be performed in the presence of a acid scavenger such as an inorganic or organic base. The base may be

potassium or sodium or quaternary ammonium carbonate, or hydroxide.

LEGENDS TO THE FIGURES

Fig. 1 shows the effect of licochalcone A on the parasitic load of the footpad of the mice infected with *Leishmania major* as described in Example 5.

Fig. 2. Five Syrian golden male hamsters weighing 50-70 g which were infected with *L. donovani* by intracardial injection of 2 x 10^7 stationary phase promastigotes. One day later the animals were injected intraperitoneally with 10 mg/kg body weight licochalcone A (100 µl in saline) for 6 days. The animals were sacrificed on day 8 and parasite load in the spleen and liver was determined by determining the growth of promastigotes from the spleen and the liver using ^{3}H-thymidine uptake by promastigotes.

EXAMPLE 1

Licochalcone A was isolated from Chinese licorice root of Glycyrrhiza species rich in licochalcone A by bioassay-guided fractionation, the bioassay being the *L. major* growth test described in Example 4.

EXAMPLE 2

This example illustrates the preparation of benzaldehydes used as starting materials.

1) Preparation of 2,4-dihydroxy-5-alkylbenzaldehydes or 2,4-dihydroxy-3,5-dialkyl-benzaldehydes

Many of these compounds are already known and can generally be prepared by reacting the corresponding 1,3-dihydroxy-4-alkylbenzene or the corresponding 1,3-dihydroxy-3,5-dialkylbenzaldehyde with hydrogen cyanide or zinc cyanide in an ether solution in the presence of hydrogen chloride followed by hydrolysis of the formed product. Alternatively, the products may be formed through a Vilsmeier-Haack reaction (see J. March, "Advanced Organic Chemistry", 1992, 542-543).

2) Preparation of 2-hydroxy-4-alk-2-enyloxy-5-alkylbenzaldehydes or 2-hydroxy-3,5-dialkyl-4-alk-2-enyloxybenzaldehydes

The appropriate 3,5-dialkyl-2,4-dihydroxybenzaldehyde or 5-alkyl-2,4-dihydroxy-benzaldehyde is selectively alkylated at the 4-hydroxy group with an alk-2-enyl bromide according to the procedure described for the synthesis of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde (see S. Khan and M. Krishnamurti in *Indian J. Chem.* **22B** (1983), 276).

3) Preparation of 2-methoxy-4-alk-2-enyloxy-5-alkylbenzaldehydes or 2-methoxy-3,5-dialkyl-4-alk-2-enyloxybenzaldehydes

The appropriate 4-alk-2-enyloxy-2-hydroxybenzaldehyde is alkylated with dimethyl sulphate according to the procedure described for the synthesis of 4-((3-methyl)but-2-enyloxy)-2-hydroxybenzaldehyde (see S. Khan and M. Krishnamurti in *Indian J. Chem.* **22B** (1983), 276).

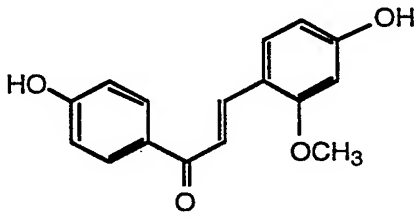
4) Preparation of 2-methoxy-5-alkyl-4-hydroxybenzaldehydes or 2-methoxy-3,5-dialkyl-4-hydroxybenzaldehydes

The appropriate 2,4-dihydroxybenzaldehyde is masked as the 4-tetrahydropyranyl-ether and alkylated with iodomethane. The procedure is described for the synthesis of 2-methoxy-4-(2-tetrahydropyranyloxy)benzaldehyde during the preparation of echinatin. The tetrahydropyranylether is cleaved by treatment with hydrochloric acid.

EXAMPLE 3

This example illustrates the preparation of bis-aromatic α,β-unsaturated ketones.

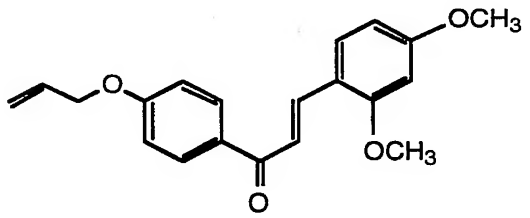
1) Preparation of echinatin



2.76 g (20 mmol) of 2,4-dihydroxybenzaldehyde, 2.7 ml (25 mmol) 3,4-dihydro-2H-pyran, and 0.1 g pyridinium tosylate were dissolved in 30 ml dichloromethane. The solution was stirred for 4 h at room temperature, washed with 20 ml 1M sodium carbonate, dried ($MgSO_4$) and concentrated *in vacuo* to give 4.3 g of an oil which according to the ^1H NMR spectrum (CD_3COCD_3) consisted of almost pure 2-hydroxy-4-tetrahydropyranyloxybenzaldehyde, δ 11.4 (1H, s, OH), 9.72 (1H, s, CHO), 7.43 (1H, d J 8.4 Hz, H6), 6.66 (1H, dd, J 8.4 Hz and 2.5 Hz. H5). 6.62 (1H, d, J 2.5 Hz, H3), 5.50 (1H, t, J 3 Hz, H2'), 3.83 (1H, bt, J 9.2 Hz, H6'), 3.65 (1H, bt, J 5.4 Hz, H6''), 2.0-1.5 (6H, m, H3'-5'). 4.4 g (20 mmol) of the above-mentioned oil, 3.20 g (80 mmol) of sodium hydroxide and 2.5 ml (40 mmol) iodomethane were slowly dissolved in 15 ml of dimethyl sulfoxide. The solution was stirred for 60 min at room temperature and added to 150 ml of water. The mixture was extracted four times with 150 ml of dichloromethane. The organic layer was washed three times with 50 ml of water, dried ($MgSO_4$) and concentrated *in vacuo* to give 4.5 g of an oil, which according to the ^1H NMR spectrum ($CDCl_3$) consisted of almost pure 2-methoxy-4-tetrahydroyranyloxybenz-aldehyde, δ 10.3 (1H, s, CHO), 7.79 (1H, d, J 8.6 Hz, H6), 6.71 (1H, dd, J 8.6 and 2.2 Hz, H5), 6.63 (1H, d, J 2.2 Hz, H3), 5.54 (1H, t, J 3 Hz, H2'), 3.90 (3H, s, CH_3O), 3.83 (1H, bt, J 9.2 Hz, H6'), 3.67 (1H, bt, J 5.4 Hz, H6''), 2.0-1.5 (6H, m, H3'-5'). 1.18 g (5 mmol) of the above-mentioned oil, 1.10 g (5 mmol) of tetrahydropyranyloxyacetophenone and 50 mg of sodium hydroxide were dissolved in 10 ml of dry ethanol. After stirring at room temperature for 12 h, 2 ml of 4M hydrochloric acid was added to the solution and stirring was continued for additional 30 min. The mixture was extracted three times with 50 ml of ether after addition of 40 ml of water. The organic phase was dried

($MgSO_4$) and concentrated *in vacuo* to give 1.2 g of a crystalline gum (overall yield 84%), which was recrystallized from ethanol-water to give reddish crystals, m.p. 220°C. The ^1H NMR spectrum was identical to that of echinatin described by T. Furuya et al, in *Tetrahedron Lett.* 2567 (1967).

2) Preparation of 2,4-dimethoxy-4'-prop-2-enyloxychalcone and analogous 2,4,4'-trioxygenated chalcones



17.6 g (0.1 mol) of 4-allyloxyacetophenone and 16.6 g (0.1 mol) of 2,4-dimethoxybenzaldehyde were under an inert dry atmosphere (argon) dissolved in 100 ml of dry ethanol (freshly distilled from sodium under argon atmosphere). The solution was added 1 g of sodium hydroxide and left under stirring for 18 h. The reaction mixture was filtered to give 29.9 g (97 %) of 2,4-dimethoxy-4'-prop-2-enyloxychalcone identical was obtained, m.p. 74.5-75°C.

^1H NMR data (200 MHz, $CDCl_3$, δ) 8.08 (d, *J* 16 Hz, H-β), 8.03 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.56 (d, *J* 16 Hz, H-α), 7.56 (d, *J* 8 Hz, H-6), 6.98 (MM'-part of an AA'MM'-system, H-3'and H-5'), 6.51 (dd, *J* 3 and 8 Hz, H-5), 6.45 (d, *J* 3 Hz, H-3), 6.03 (ddt, *J* 15, 10 and 4 Hz, -C<u>H</u>=), 5.41 (d, *J* 15 Hz, =CH<u>H</u>), 5.28 (d, *J* 10 Hz, =C<u>H</u>H), 4.59 (d, *J* 4 Hz, -CH_2-), 3.89 and 3.85 (s, CH_3-O).

^{13}C NMR data (50 MHz, $CDCl_3$, δ) 189.3, 162.9, 162.0, 160.3, 139.6, 132.6, 131.7, 130.8, 130.6, 120.0, 118.0, 117.2, 114.4, 105.4, 98.4, 68.8, 55.5, 55.4.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.12, H 6.30

In an analogous manner, but substituting 2,4-dimethoxybenzaldehyde with an appropriate benzaldehyde, the following chalcones are prepared:

2,4-Diethoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,

2,4-diethoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

In an analogous manner, but by appropriate substitution of 2,4-dimethoxybenzaldehyde and substitution 4-(2-propenyloxy)acetophenone with 4-methoxy-, 4-ethoxy- or 4-propoxyacetophenone, respectively, the following chalcones are prepared:

2,4-dimethoxy-4'-methoxychalcone,
2,4-diethoxy-4'-methoxychalcone,
2,4-di-n-propoxy-4'-methoxychalcone,
2,4-diisopropoxy-4'-methoxychalcone,
2,4-di-n-butoxy-4'-methoxychalcone,
2,4-di-t-butoxy-4'-methoxychalcone,
2,4-dimethoxy-5-methyl-4'-methoxychalcone,

2,4-diethoxy-5-methyl-4'-methoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-methoxychalcone,
2,4-diisopropoxy-5-methyl-4'-methoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-methoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-methoxychalcone,
2,4-dimethoxy-5-propyl-4'-methoxyhalcone,
2,4-diethoxy-5-propyl-4'-methoxychalcone,
2,4-di-n-propoxy-5-propyl-4'-methoxychalcone,
2,4-diisopropoxy-5-propyl-4'-methoxyhalcone,
2,4-di-n-butoxy-5-propyl-4'-methoxychalcone,
2,4-di-t-butoxy-5-propyl-4'-methoxyhalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methoxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,

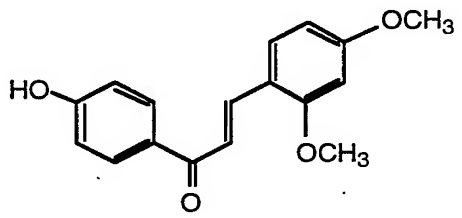
2,4-dimethoxy-4'-ethoxychalcone,
2,4-diethoxy-4'-ethoxychalcone,
2,4-di-n-propoxy-4'-ethoxychalcone,
2,4-diisopropoxy-4'-ethoxychalcone,
2,4-di-n-butoxy-4'-ethoxychalcone,
2,4-di-t-butoxy-4'-ethoxychalcone,
2,4-dimethoxy-5-methyl-4'-ethoxychalcone,
2,4-diethoxy-5-methyl-4'-ethoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-ethoxychalcone,
2,4-diisopropoxy-5-methyl-4'-ethoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-ethoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-ethoxychalcone,

2,4-dimethoxy-5-propyl-4'-ethoxyhalcone,
2,4-diethoxy-5-propyl-4'-ethoxychalcone,
2,4-di-n-propoxy-5-propyl-4'-ethoxychalcone,
2,4-diisopropoxy-5-propyl-4'-ethoxyhalcone,

2,4-di-n-butoxy-5-propyl-4'-ethoxychalcone,
2,4-di-t-butoxy-5-propyl-4'-ethoxyhalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,

2,4-dimethoxy-4'-propoxychalcone,
2,4-diethoxy-4'-propoxychalcone,
2,4-di-n-propoxy-4'-propoxychalcone,
2,4-diisopropoxy-4'-propoxychalcone,
2,4-di-n-butoxy-4'-propoxychalcone,
2,4-di-t-butoxy-4'-propoxychalcone,
2,4-dimethoxy-5-methyl-4'-propoxychalcone,
2,4-diethoxy-5-methyl-4'-propoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-propoxychalcone,
2,4-diisopropoxy-5-methyl-4'-propoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-propoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-propoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone and
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone.

3) Preparation of 2,4-dimethoxy-4'-hydroxychalcone



326 mg of 2,4-dimethoxy-4'-allyloxychalcone was dissolved in 5 ml of methanol, 1 ml water, palladium on charcoal (10%, 100 mg), and 100 mg of p-toluene-sulfonic acid were added, and the mixture was refluxed for 24 h. The reaction mixture was filtered and poured into a mixture of 5 ml of a 10% aqueous solution of sodium bicarbonate and 5 ml of a saturated aqueous sodium chloride solution. The solution was extracted with 10 ml of ethyl acetate and concentrated to give 380 mg which were chromatographed over silica gel 60 (Merck 0.063-0.200 mm, 25 g, eluent petroleum ether-ethyl acetate 9:1) to give 130 mg of yellow crystals which were recrystallized from methanol to give 70 mg of 2,4-dimethoxy-4'-hydroxychalcone, m.p. 165-166°C.

^{1}H-NMR data (200 MHz, CD_3CN-DMSO-d_6, δ): 7.98 (AA-part of an AA´MM´-system, H-2´and H-6´), 7.96 (d, *J* 15 Hz, H-β), 7.72 (d, *J* 7 Hz, H-6), 7.65 (d, *J* 15 Hz, H-α), 6.91 (MM´-part of an AA´MM´-system, H-3´and H-5´), 6.59 (dd, *J* 7 and 2 Hz, H-5), 6.57 (d, *J* 2 Hz, H-3), 3.90 and 3.83 (CH_3-O).

^{13}C NMR data (50 MHz, CD_3CN-DMSO-d_6, δ) 187.5, 138.8, 157.8, 117.5, 161.1, 99.2, 162.9, 106.8, 13.0, 131.7, 116.2, 164.0, 116.2, 131.7, 56.4, 56.2.

Calc. for $C_{17}H_{16}O_4$ C 71.82, H 5.67. Found: C 71.48, H 5.82.

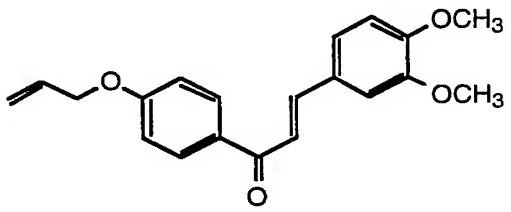
4) Preparation of 2,4-dimethoxy-4'-hydroxychalcone and analogous 2,4-dialkoxy-4'-hydroxychalcones

A solution of 2.8 g (12.7 mmol) 4-(2-tetrahydropyranyl)oxyacetophenone, 1.35 g (12.7 mmol) 2,4-dimethoxybenzaldehyde and 127 mg sodium hydroxide in 20 ml dry ethanol was left for 12 h with stirring at room temperature. To the reaction mixture was added 10 ml of 4M hydrochloric acid and the mixture was stirred for additional 30 min. The mixture was filtered and the precipitate recrystallized from ethanol-water to give 2 g (70%) 2,4-dimethoxy-4'-hydroxychalcone, m.p. 167-168°C. The ^{1}H NMR spectrum of the obtained product was superimposable to the spectrum of previously prepared 2,4-dimethoxy-4'-hydroxychalcone.

In an analogous manner, but substituting the 2,4-dimethoxybenzaldehyde with an appropriate benzaldehyde, the following chalcones are prepared:

2,4-Dimethoxy-4'-hydroxychalcone,
2,4-diethoxy-4'-hydroxychalcone,
2,4-di-n-propoxy-4'-hydroxychalcone,
2,4-diisopropoxy-4'-hydroxychalcone,
2,4-di-n-butoxy-4'-hydroxychalcone,
2,4-di-t-butoxy-4'-hydroxychalcone,
2,4-dimethoxy-5-methyl-4'-hydroxychalcone,
2,4-diethoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-methyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-methyl-4'-hydroxychalcone,
2,4-di-t-butoxy-5-methyl-4'-hydroxychalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-hydroxychalcone and
2,4-di-t-butoxy-5-prop-2-enyl-4'-hydroxychalcone

5) Preparation of 3,4-dimethoxy-4'-prop-2-enyloxychalcone and analogous 3,4,4'-trioxygenated chalcones



1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 3,4-dimethoxybenzaldehyde were under a dry inert atmosphere (argon) dissolved in 10 ml dry ethanol and the solution was stirred for 18 h. The solution was filtered to give 3.0 g (99 %) of 3,4-dimethoxy-4´-prop-2-enyloxychalcone which was recrystallized from ethanol, m.p. 74.5-75°C.

^{1}H NMR data (200 MHz, CD_3CN, δ) 8.06 (AA'-part of an AA´MM´-system, H-2´ and H-6´), 7.70 (d, *J* 15 Hz, H-β), 7.58 (d, *J* 15 Hz, H-α), 7.33 (d, *J* 2 Hz, H-2), 7.25 (dd, *J* 2 and 8 Hz, H-6), 7.00 (MM´-part of an AA´MM´-system, H-3´ and H-5´), 6.91 (d, *J* 8 Hz, H-5), 6.04 (m, =C<u>H</u>-), 5.42 (m, =CH<u>H</u>), 5.28 (m, =C<u>H</u>H), 4.60 (m, -CH_2-), 3.87 and 3.83 (s, CH_3).

^{13}C NMR data (50 MHz, CD_3CN, δ) 188.3, 161.8, 151.0, 148.9, 142.9, 132.7, 130.8, 130.1, 127.4, 122.8, 119.1, 116.8, 113.9, 110.9, 109.9, 68.1, 54.9, 54.8.

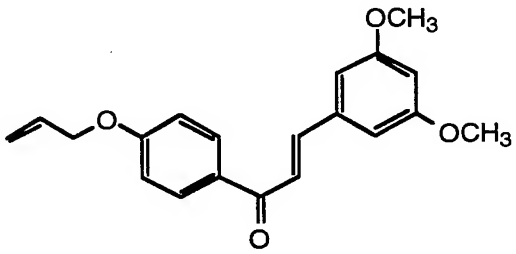
Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.10, H 6.24.

In an analogous manner, but substituting the 3,4-dimethoxybenaldehyde with an appropriate 3,4-dialkoxybenzaldehyde, the following chalcones are prepared:

3,4-Diethoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy-chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone, and
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

6) Preparation of 3,5-dimethoxy-4'-prop-2-enyloxychalcone and analogous 3,5,4'-trioxygenated chalcones



1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 3,5-dimethoxybenzaldehyde were dissolved in 10 ml of dry freshly distilled ethanol under an inert atmosphere (argon), and the solution was admixed with 100 mg of sodium hydroxide and left under stirring for 18 h. The reaction mixture was filtered to give 2.96 g (99 %) of 3,5-dimethoxy-4'-prop-2-enyloxychalcone which was recrystallized from methanol, m.p. 88.5-90°C.

^{1}H NMR data (200 MHz, CD_3CN, δ) 8.06 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.68 (d, *J* 15 Hz, H-β), 7.62 (d, *J* 15 Hz, H-α), 7.01 (MM'-part of an AA'MM'-system, H-3'and H-5') 6.88 (d, *J* 2 Hz, H-2 and H-6) 6.53 (t, *J* 2 Hz, H-4), 6.10 (m, =C<u>H</u>-), 5.39 (m, =CH<u>H</u>), 5.28 (m, =C<u>H</u>H), 4.61 (m, -CH_2-), 3.80 (s, CH_3O).

^{13}C NMR data (50 MHz, CD_3CN, δ): 187.4, 161.9, 160.6, 142.6, 136.6, 132.6, 130.5, 130.3, 122.0, 116.8, 113.9, 116.8, 113.9, 105.8, 101.9, 68.2, 54.7.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.02, H 6.24

In an analogous manner, but substituting the 3,5-dimethoxybenaldehyde with an appropriate 3,5-dialkoxybenzaldehyde, the following chalcones are prepared:

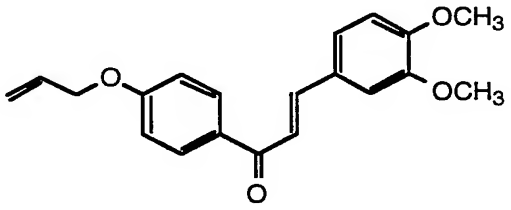
3,5-diethoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,

3,5-dimethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

3,5-dimethoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,

3,5-di-n-butoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

7) Preparation of 3,4-dimethoxy-4'-prop-2-enyloxychalcone and analogous 3,4,4'-trioxygenated chalcones



1.76 g (10 mmol) of 4-allyloxyacetophenone and 1.66 g (10 mmol) of 3,4-dimethoxybenzaldehyde were under a dry inert atmosphere (argon) dissolved in 10 ml dry ethanol and the solution was stirred for 18 h. The solution was filtered to give 3.0 g (99 %) of 3,4-dimethoxy-4'-prop-2-enyloxychalcone which was recrystallized from ethanol, m.p. 74.5-75°C.

^{1}H NMR data (200 MHz, CD_3CN, δ) 8.06 (AA'-part of an AA'MM'-system, H-2' and H-6'), 7.70 (d, *J* 15 Hz, H-β), 7.58 (d, *J* 15 Hz, H-α), 7.33 (d, *J* 2 Hz, H-2), 7.25 (dd, *J* 2 and 8 Hz, H-6), 7.00 (MM'-part of an AA'MM'-system, H-3' and H-5'), 6.91 (d, *J* 8 Hz, H-5), 6.04 (m, =C<u>H</u>-), 5.42 (m, =CH<u>H</u>), 5.28 (m, =C<u>H</u>H), 4.60 (m, -CH_2-), 3.87 and 3.83 (s, CH_3).

^{13}C NMR data (50 MHz, CD_3CN, δ) 188.3, 161.8, 151.0, 148.9, 142.9, 132.7, 130.8, 130.1, 127.4, 122.8, 119.1, 116.8, 113.9, 110.9, 109.9, 68.1, 54.9, 54.8.

Calc. for $C_{20}H_{20}O_4$: C 74.06, H 6.21. Found: C 74.10, H 6.24.

In an analogous manner, but substituting 3,4-dimethoxybenzaldehyde with an appropriate benzaldehyde, the following chalcones are prepared:

3,4-diethoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

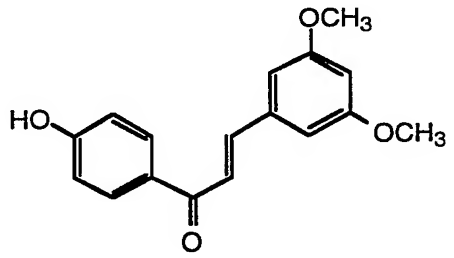
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone, and
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

In an analogous manner, but by appropriate substitution of 2,4-dimethoxybenzaldehyde and substitution of 4-(prop-2-enyloxy)acetophenone with 4-propoxyacetophenone, the following chalcones are prepared:

3,4-dimethoxy-4'-propoxychalcone,
3,4-diethoxy-4'-propoxychalcone,
3,4-di-n-propoxy-4'-propoxychalcone,
3,4-diisopropoxy-4'-propoxychalcone,
3,4-di-n-butoxy-4'-propoxychalcone,
3,4-di-t-butoxy-4'-propoxychalcone,
3,4-dimethoxy-2-methyl-4'-propoxychalcone,
3,4-diethoxy-2-methyl-4'-propoxychalcone,
3,4-di-n-propoxy-2-methyl-4'-propoxychalcone,
3,4-diisopropoxy-2-methyl-4'-propoxychalcone,
3,4-di-n-butoxy-2-methyl-4'-propoxychalcone,
3,4-di-t-butoxy-2-methyl-4'-propoxychalcone,
3,4-dimethoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-diethoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-n-propoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-diisopropoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-n-butoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-t-butoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-dimethoxy-5-propyl-4'-propoxychalcone,
3,4-diethoxy-5-propyl-4'-propoxychalcone,
3,4-di-n-propoxy-5-propyl-4'-propoxychalcone,
3,4-diisopropoxy-5-propyl-4'-propoxychalcone,
3,4-di-n-butoxy-5-propyl-4'-propoxychalcone,
3,4-di-t-butoxy-5-propyl-4'-propoxychalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,

3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone, and
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone.

8) Preparation of 3,5-dimethoxy-4'-hydroxychalcone and analogous 3,5,4'-trioxygenated chalcones



2.24 g (10 mmol) 4-methoxyethoxymethoxyacetophenone, 1.6 g (10 mmol) 3,5-dimethoxybenz-aldehyde and 100 mg sodium hydroxide were dissolved in 10 ml of dry ethanol and the solution was left overnight at room temperature. Upon addition of 2 ml 4M hydrochloric acid, the solution was concentrated to half volume *in vacuo* and the residue was extracted twice with 10 ml of ether. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give 3.22 g of an oil from which 1.96 g (52%)of 3,5-dimethoxy-4-methoxyethoxymethoxychalcone was isolated by column chromatography over silica gel (350 g) using as an eluent petroleum ether-ethyl acetate- glacial acetic acid (80:20:0.5) to which increasing amounts of ethyl acetate was added. 0.37 g (1 mmol) of the methoxyethoxymethoxyether was dissolved in 2 ml of ethanol and added 0.1 ml 4M hydrochloric acid and the mixture was refluxed for 15 min. To the solution was added 0.2 ml 1M sodium carbonate and the solution was concentrated *in vacuo*. 0.27 g (100%) of 3,5-dimethoxy-4'-hydroxychalcone was isolated from the residue by column chromatography over silica gel (25 g) using as an eluent petroleum ether-ethyl acetate (3:2). The product was recrystallized from methanol-water to give slightly yellow crystals, m.p. 123-127°C.

^1H NMR (CD_3CN, δ) 8.03 (2H, AA'-part of an AA'MM'-system, H2' and H6'), 7.74 (1H, d, *J* 15 Hz, Hβ), 7.63 (1H, d, *J* 15 Hz, Hα), 7.0-6.85 (4H, m, H3', H5', H2 and H6), 5.55 (1H, t, *J* 2 Hz, H4), 3.84 (6H, s, CH_3O).

^{13}C NMR (CD_3CN, δ) 190, 161.8, 160.6, 142.2, 137.2, 130.5, 129.1, 122.1, 114.8, 105.7, 101.7, 54.6.

In an analogous manner, but with appropriate substitution of the starting materials, the following chalcones are prepared:

3,5-dimethoxy-4'-hydroxychalcone,
3,5-diethoxy-4'-hydroxychalcone,
3,5-di-n-propoxy-4'-hydroxychalcone,
3,5-diisopropoxy-4'-hydroxychalcone,
3,5-di-n-butoxy-4'-hydroxy)chalcone,
3,5-di-t-butoxy-4'-hydroxy)chalcone,

3,5-dimethoxy-2-methyl-4'-hydroxychalcone,
3,5-diethoxy-2-methyl-4'-hydroxychalcone,
3,5-di-n-propoxy-2-methyl-4'-hydroxychalcone,
3,5-diisopropoxy-2-methyl-4'-hydroxy)chalcone,
3,5-di-n-butoxy-2-methyl-4'-hydroxychalcone,
3,5-di-t-butoxy-2-methyl-4'-hydroxychalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-diethoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-hydroxychalcone,
3,5-dimethoxy-2-propyl-4'-hydroxychalcone,
3,5-diethoxy-2-propyl-4'-hydroxychalcone,
3,5-di-n-propoxy-2-propyl-4'-hydroxychalcone,
3,5-diisopropoxy-2-propyl-4'-hydroxychalcone,
3,5-di-n-butoxy-2-propyl-4'-hydroxychalcone,
3,5-di-t-butoxy-2-propyl-4'-hydroxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4' hydroxychalcone,

3,5-dimethoxy-4-methyl-4'-hydroxy)chalcone,
3,5-diethoxy-4-methyl-4'-hydroxychalcone,
3,5-di-n-propoxy-4-methyl-4'-hydroxychalcone,

3,5-diisopropoxy-4-methyl-4'-hydroxychalcone,
3,5-di-n-butoxy-4-methyl-4'-hydroxychalcone,
3,5-di-t-butoxy-4-methyl-4'-hydroxychalcone,
3,5-dimethoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-diethoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-di-n-propoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-diisopropoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-di-n-butoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-di-t-butoxy-4-prop-2-enyl-4'-hydroxychalcone,
3,5-dimethoxy-4-propyl-4'-hydroxychalcone,
3,5-diethoxy-4-propyl-4'-hydroxychalcone,
3,5-di-n-propoxy-4-propyl-4'-hydroxychalcone,
3,5-diisopropoxy-4-propyl-4'-hydroxychalcone,
3,5-di-n-butoxy-4-propyl-4'-hydroxychalcone,
3,5-di-t-butoxy-4-propyl-4'-hydroxychalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-hydroxy)chalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-hydroxy)chalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone and
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone.

EXAMPLE 4

Effect of a number of bis-aromatic α,β-unsaturated ketones on *L. major* promastigotes from 4-days cultures on *Plasmodium falciparum* growth *in vitro* and on human lymphocyte proliferation response to PHA

Materials and methods

Parasite cultures. A WHO reference vaccine strain of *L. major* originally isolated from a patient in Iran and a Kenyan strain of *L. donovani* (MHOM(/KE/85/NLB 274) were cultured in medium 199 containing 0.02 mg/ml gentamycin, 25 mM Hepes, 4 mM L-glutamine, and 20% heat inactivated fetal calf serum (FCS). Incubation was carried out at 26°C. Promastigotes were harvested on day 3 and 6 of the culture and used for

the parasite growth inhibition.

Drugs. Licochalcone A and analogues thereof, prodrugs thereof and a heterocyclic chalcone.

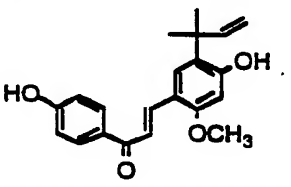
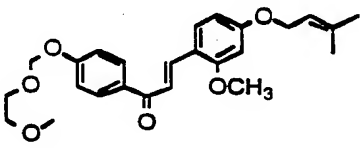
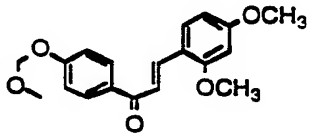
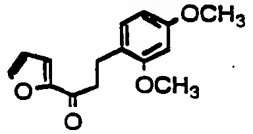
Effect on *Leishmania* promastigotes. The effect of the compounds on promastigotes were assessed by a method similar to the one described by Pearson et al., by incubating promastigotes ($3x10^6$/ml) at 26°C for 2 hrs in the presence of a given compound or the medium alone in 96 wells flat bottom microtiter plates. Following incubation, 100 µCi of ^{3}H-thymidine was added to each well and further incubated for 18 hrs. Promastigotes were then harvested on filter paper by means of a cell harvester, extensively washed with distilled water and counted in a scintillation counter. The promastigotes were also counted microscopically and their flagellar motility was assessed.

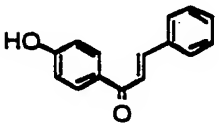
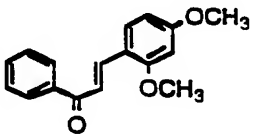
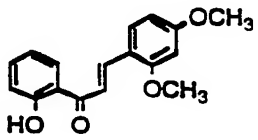
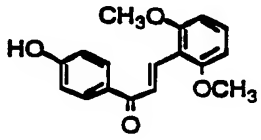
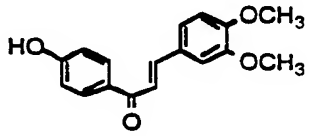
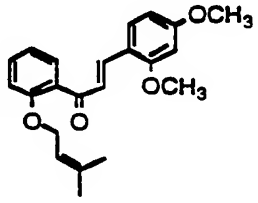
Testing of the effect of drugs on the *in vitro* growth of *Plasmodium falciparum* parasites. The experiments in which compounds were tested for their ability to inhibit parasite growth were performed by a modification of the method originally described by Jensen et al. (1982). Fifty µl of parasitized erythrocytes (parasitemia approximately 1%) in a concentration of $5x10^8$/ml and 50 µl of RPMI medium containing different concentrations of the test compound was added to each well of a 96 well flat-bottomed microtiter plate. The cultures were then incubated for 48 hours, 24 hours before termination of the culture adding 20 µl of 3-H-hypoxanthine (40 uCi/ml) was added to each well. The cultures were then harvested onto glass fiber filters using a Skatron cell harvester, and the incorporation of 3-H-hypoxanthine into the DNA of dividing parasites was determined by liquid scintillation spectrometry. Control cultures with uninfected erythrocytes and infected erythrocytes in RPMI medium without test compounds were always performed in parallel to the test cultures. In some experiments thin smears of parasite cultures were stained by Giemsa and examined under microscope (x 1000). The test compounds described above, were diluted in RPMI medium immediately before use. In the experiments, chloroquine phosphate was used as a positive control as a drug known to inhibit parasite growth.

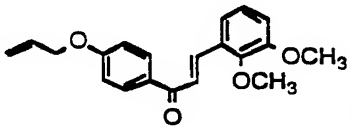
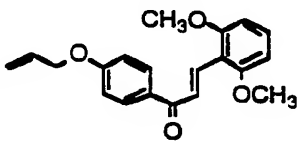
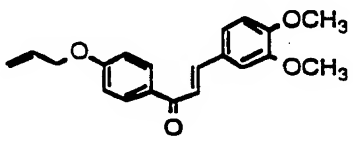
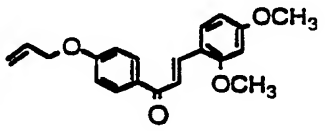
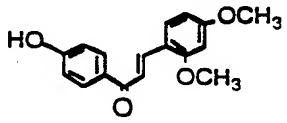
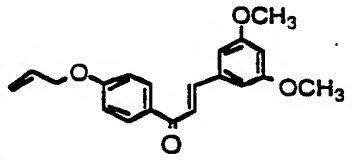
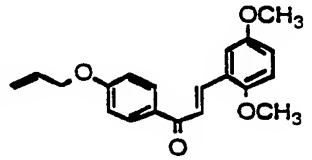
Lymphocyte proliferation. Human blood mononuclear cells (BMNC) from heparinized blood were isolated by metrizoate sodium-Ficoll density gradient centrifugation, washed 3 times in RPMI 1640 medium supplemented with 5% FCS and with 400 IU of penicillin plus 400 µg/ml streptomycin. BMNC were resuspended in the medium and cultured in triplicate, $0.63x10^5$/ml and 160 µl per vial, in round-bottom microtiter plates with 20 µl of various concentrations of licochalcone A. Immediately prior to incubation, optimum concentrations of the mitogen phytohaemagglutinin (PHA) and the antigen purified protein derivative of tuberculin (PPD) were added to the cultures in a volume of 20 µl. Unstimulated control

cultures were always included. Cultures were incubated for 3 or 7 days. The degree of lymphocyte proliferation was estimated by ^{3}H-thymidine (1 µCi per well) addition 24 h before the cells were harvested on glass fiber filters by means of a harvesting machine), and ^{3}H-thymidine incorporation was measured in a liquid scintillation counter. For each set of triplicate values, the median was recorded. Unstimulated cultures were always included as controls.

Table 4.1. The effect of chalcones on *L. major* promastigotes from 4-days cultures, on *P. falciparum* growth *in vitro*, and on human lymphocyte proliferation response to PHA. The upper figures are percentage inhibition of human promastigotes, the middle figures (*italic*) are inhibition of malaria parasites, the lower figures (**bold**) are inhibition of lymphocytes. When standard deviation is given, more than five experiments have been performed.

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	**92** *98.0±0.0* **39.7±5.4**	**64** *98.0±0.0* **20.8±4.8**	**25** *69.0±4.0* **7.7±5.7**	**12** *39.0* **4.7±5.6**
	61.5 **28.3±11.5**	33.1 **14.1±7.8**	14 **8.1±11.2**	11.5 **0±11.0**
	65.4±16.1 **15.4±18.7**	50.2±13.0 **11.2±15.4**	27.6±10.9 **5.1±11.9**	22.8±9.7 **0±4.5**
	89.6 **41.9**	55.1 **33.6**	9.4 **11.7**	9.5 **3.6**

Formula	10 µg/ml	5 µg/ml	1 µg/ml	0.5 µg/ml
	90.1±6.5 *95.6±1,.* **17.0±18.3**	73.4±15.3 *64.3±8.5* **10.2±13.0**	37.3±13.6 *40.5±11.0* **3.6±6.0**	26.4±6.5 *29.9±10.7*
	95.5±3.8 *81.8* **3.1±15.4**	77.8±11.3 *35.6* **2.0±13.2**	27.0±13.7 *14.7* **9.2±10.8**	14.5±11.6 *0* **9.1±14.0**
	98.3±1.0 *67.9* **0±7.0**	89.4±13.8 *23.4* **2.6±10.3**	37.6±18.3 *0.8* **5.5±12.3**	19.2±18.1 *0* **3.0±10.3**
	93.1±4.6 *90.6* **4.4±27.9**	77.5±4.3 *71* **2.3±18.4**	21.5±10.6 *16.6* **3.3±12.2**	11.4±9.2 *0* **0±11.5**
	80 *73.4* **27.4±26.8**	65.6 *39.9* **5.2±9.9**	34.3 *35.1* **0.1±8.71**	17.8 *21.0* **0±6.62**
	83.2 **35.9**	49.0 **8.7**	1.8 **6.6**	4.3 **7.1**

Formula	10 ug/ml	5 ug/ml	1 ug/ml	0.5 ug/ml
	98.1±1.0 *73.1* **13.8±14.6**	82.1±13.6 *68.2* **8.8±20.1**	20.7±17.1 *17.2* **14.2±20.5**	13.9±12.9 *0* **10.8±19.3**
	91.1±4.1 *65.7* **0±11.6**	63.7±24.2 *39.7* **0±10.2**	16.3±12.4 *27.4* **1.3±10.2**	11.3±9.8 *0* **1.0±11.4**
	90.8±9.7 *68* **15.6±25.0**	70.4±15.2 *22.8* **0±8.7**	30.4±23.2 *11.4* **0±9.3**	15.2±11.3 *2.9* **0±10.6**
	85.4 *97.3* **5.5±18.8**	71.4 *74.4* **5.9±9.9**	62.8 *36.5* **1.7±11.5**	35.4 *31.1* **0±9.8**
	90.9±7.4 *64.2* **4.0±15.1**	66.9±15.9 *31.6* **2.4±11.1**	29.0±22.2 *14.8* **6.3±14.9**	20.0±20.6 *2.2* **1.5±11.6**
	98.3±0.9 *98.6* **25.4±16.3**	82.8±10.0 *74.3* **4.6±8.4**	27.6±23.3 *15.3* **0±7.8**	10.6±10.7 *13.8* **0±5.9**
	89.4±10.2 *80* **2.3±12.7**	63.0±15.1 *35* **4.3±14.8**	15.6±15.1 *0* **12.6±16.6**	10.0±9.7 *0* **5.7+13.0**

Conclusion

The data in Table 4.1 indicate the importance of oxygenation in the, e.g. 2,4-, 3,4- or 4'-hydroxy-3,5-position for obtaining compounds which preferentially inhibit thymidine uptake into the parasites. The *in vitro* results reported above confirm the hypothesis that the unsaturated α,β-position is of importance for the activity. This is substantiated by a very low activity shown by 1-(4-hydroxyphenyl)-3-(2,4-dimethoxy)phenyl-2-propan-1-one. The pattern shown by the results indicates that one of the mechanisms of action might be an alkylation of the target biomolecule by the α,β-unsaturated ketones. It can be shown that licochalcone A is able to react with a thiol-containing peptide, by which reaction a nucleophilic thiol group is added to the α,β-double bond. The principle is well known from the anti-cancer activity of α-methylene sesquiterpene lactones. One such α-methylene sesquiterpene lactone has been tested in the *in vitro* model and has been found to be extremely active against *Leishmania* parasites *in vitro*, but at the same time also to show extreme toxicity on human lymphocytes (data not shown). Thus, the substituents in the chalcone skeleton contribute to the selectivity of these compounds. The effect of the substituents can also be seen from the fact that chalcone in itself also has a very high activity against *Leishmania* parasites but is also extremely toxic against human lymphocytes (data not shown), whereas, as is evident from the above data, e.g. 2,4-, 3,4- or, or 4'-hydroxy-3,5-oxygen substituted chalcones show considerable selectivity for a number of chalcones.

EXAMPLE 5

Effect of licochalcone A on the *in vivo* growth of *L. major*

Materials and Methods

Mice. BALB/c female mice aged eight weeks old were used throughout.

Parasite cultures. The WHO reference vaccine strain of *L. major* originally isolated from a patient in Iran and a Kenyan strain of *L. donovani* (MHOM(/KE/85/NLB 274) were cultured as described in Example 4. For animal infection, mice received s.c. injections (in 0.05 ml of PBS) in the left hind footpad with $1x10^7$ stationary phase promastigotes.

Footpad lesions were measured and expressed as footpad thickness increase (in mm). The footpad thickness of mice was measured before infection and every 3 days after 7 days of infection. From 7 days of infection, mice received licochalcone A injections i.p. once a day. After 42 days of licochalcone A injection, some of the mice were killed and the footpads, spleens and livers removed. The parasite loads in the footpads and

livers were estimated by a modification of the method described by Liew et al. using 3-H-thymidine uptake. The results were expressed as cpm. The footpads, spleens and livers impression was also estimated.

Drugs. One mg of licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and then 980 µl of medium 199 was added an the resulting mixture was stored at -20°C before use.

Results

Table 5.1. Effect of licochalcone A on the parasitic load of the footpad of the mice infected with *L. major*. The results are from 2 mice from each group and are given as mean x10^3 cpm of ^{3}H-thymidine uptake.

Group	mean
1) 100 µg i.p.	89.0
2) 50 µg i.p.	58.0
3) Buffer	357.6

Table 5.2. Effect of licochalcone A on the parasitic load of the liver of the mice infected with *L. major*. The results are from 2 experiments and are given as mean x10^3 cpm of ^{3}H-thymidine uptake.

Group	mean
1) 100 µg i.p.	12.8
2) 50 µg i.p.	11.7
3) Buffer	184.0

Table 5.3. Effect of licochalcone A on *L. major* parasite in the footpad, spleen and liver of the mice infected with *L. major*. The results are given as amastigotes findings on the impression smear.

Group	Footpad	Spleen	Liver
1) 100 µg i.p.	+	-	-
2) 50 µg i.p.	+	-	-
3) Buffer	+++	++	++

- no parasite amastigotes detected in 5 fields of microscopical vision
+: parasite amastigotes from 5 fields of microscopical vision < 100
++: parasite amastigotes from 5 fields of microscopical vision=100-500
+++: parasite amastigotes from 5 fields of microscopical vision=500-1000

Fig. 1 shows the effect of licochalcone A on footpad thickness increase (swelling) in BALB/c mice infected with *L. major*, expressed in mm.

Conclusion

These data clearly demonstrate that intraperitoneal administration of licochalcone A prevents lesion development in mice caused by *Leishmania* infection.

EXAMPLE 6

Effect of licochalcone A on *L. donovani* infection in hamsters

Animals. Male Syrian golden hamsters (*Mesocricetus auratus*), 50-70 g body weight, were used throughout.

Parasite. *L. donovani* (MHOM/KE/85/NLB 439) promastigotes were used.

Drugs. Licochalcone A was dissolved in 20 µl of 99% (v/v) ethanol, and the 980 µl of medium 199 was added, and the resulting mixture was stored at -20°C.

Animals were intracardially inoculated with 2 x 10^7 *L. donovani* promastigotes in 0.1 ml medium 199 (Day 0). One hour later, one of the animals was killed. The liver and the spleen were weighed. The liver and the spleen impression smears were made. After air-drying, the impression smears were fixed with water-free methanol and

stained with Giemsa. Five of the animals were treated (i.p.) with licochalcone A (10 mg/kg body weight two times per day) from Day+1 to Day+7. Another five animals were treated with 0.85% NaCl. The animals were killed on Day+8. The liver and spleen were weighed, and the liver and the spleen impression smears were made. The number of the parasite in the liver and the spleen were counted under microscope. The spleen of the animals were cut into very small pieces, cultured in 15 ml of the culture medium at 26°C overnight and the parasite load was determined by ^{3}H-thymidine uptake as described in Example 4 herein.

Results and conclusions

As shown in Fig. 2, the parasite load both in the liver and the spleen of animals receiving intraperitoneal injections of 10 mg per kg body weight licochalcone A two times per day for 7 days was reduced to almost undetectable levels.

The *in vivo* inhibitory effect of licochalcone A on *L. donovani*, the causative agent of the fatal visceral leishmaniasis, is quite promising especially in the light of resistance development against antimonials, the only antileishmanial drug in use.

EXAMPLE 7

Effects of licochalcone A and some oxygenated chalcones on the *in vivo* growth of malaria parasites

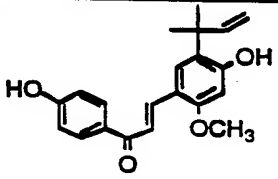
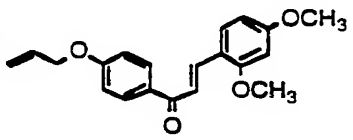
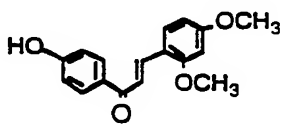
Materials and Methods

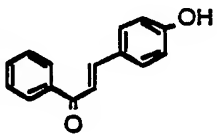
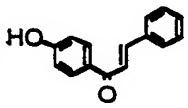
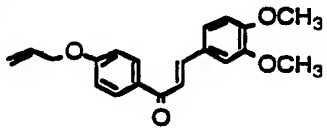
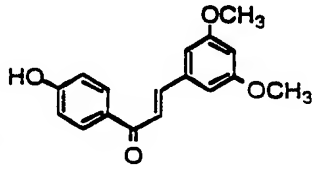
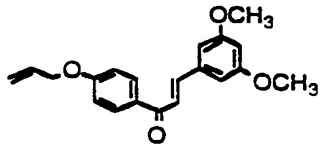
Mice. BALB/c female mice aged eight weeks were used.

Parasites. The *Plasmodium sp.* causing malaria in humans can only infect certain primates. Therefore it has not been possible to determine whether licochalcone A or some of the analogues thereof inhibit parasite multiplication of human malaria parasites *in vivo*. However, there are several *Plasmodium sp.* that infect rodents. These systems have earlier been used to test the ability of drugs to inhibit malaria infections *in vivo*. In the experiments described below mice were infected with*P. yoelii* YM strain (Table 7.1) and were compared to the outcome of infection in untreated control animals and in animals treated with licochalcone A. The parasites were maintained by passage through BALB/c mice, and the animals were infected by injection of infected erythrocytes obtained from mice with a parasitemia of approximately 40%. The animals were injected intraperitoneally with either 1×10^{6} parasitized erythrocytes diluted in 0.9% NaCl and in a final volume of 0.2 ml. The day of infection was termed day 0.

Assessment of effect. The outcome of infection was assessed microscopically by examination of Giemsa stained blood films. The load of infection (the parasitemia) was calculated as the percentage of infected erythrocytes of the total number of erythrocytes.

Table 7.1. Effect of licochalcone A and analogues thereof in BALB/c mice infected with *P. yoelii* YM strain. Parasitemia in mice with licochalcone A or an analogue thereof. The treatment was initiated 24 hours after infection with 1 x 10^6 parasites/mouse, and the mice received i.p. various concentrations of each compound as indicated below for a total of 5.5 days. The symbol # indicates number of animals.

Formula	Dose mg/kg	Parasitemia on day after infection Figures in parentheses indicate number of dead animals							#
		6	8	10	12	14	16	18	
	40	1.3	13	35	78	(2)			2
	20	0.0	1.3	6.5	25	6.5	0.3	0.0(0)	2
	10	2.3	19	41	87	(2)			2
	80	56	83(2)	(5)					5
	60	37	67	(5)					5
	40	0.9	3.1	15	33	16(3)	3.6(3)	2.5(5)	9
	20	0.9	4.3	12	31	12	2.8	0.1	4
	10	0.8	2.8	9.5	24	19	5	0.1	4
	5	0.2	2.9	13	24(1)	17(2)	15(2)	15(2)	5
	1	0.8	8.6	17	22(2)	17(2)	31(2)	20(2)	5
	80	(5)							5
	60	46	70(1)	(5)					5
	40	3.5	8	37	28(3)	15(3)	9(3)	2.5(3)	9
	20	0.8	3.5	11	27	19	7.2	0.6	4
	10	3.5	4.5	13	31	57	24(1)	8.3(1)	4
	5	0.8	1.5	5.4	14	13	2.2	0.2	5
	1	0.9	7.2	21	41	59	56	45(1)	5

Formula	Dose mg/kg	Parasitemia on day after infection Figures in parentheses indicate number of dead animals							#
		6	8	10	12	14	16	18	
	80	2.3	17						3
	40	0	1.5						3
	20	2.7	22						3
	10	3.6	36						3
	80	2.2	9	35.3	57	49(2)	11(2)	1(2)	3
	40	0.5	3.7	15	33	19	4.7	0	3
	20	1.3	7.3	24	49	23	7	0.3	3
	10	1.1	6.3	22	45	26	9.3	0.8	3
	80	1.0	8.0						3
	40	0.8	8.8						3
	20	1.7	17						3
	10	3.3	24						3
	80	2.2	9	14	48	28	16	3.7	3
	40	3.3	22	39	66(1)	73(1)	38(1)	25(1)	3
	20	3.7	32	47	67(1)	71(1)	35(1)	28(1)	3
	10	3.2	33	58(1)	71(1)	73(1)	65(1)	50(1)	3
	80	16	28(3)	48(3)	28(4)	11(4)	2(4)	0(4)	5
	40	9	10(1)	17(1)	25(1)	9(1)	1.5(1)	0(1)	5
	20	1.2	7	18	25	8	1.4	0(0)	5
	10	19	23(3)	50(3)	29(4)	10(4)	2(4)	0(4)	5

Conclusion

Thus, the data presented in Table 7.1 indicate that:

1. Licochalcone A and some of the analogues thereof are able to completely clear the infection of *P. yoelii* in mice.

2. The dose range protective for the mice for some of the analogues is fairly broad. It is interesting to note that the 4'-hydroxy-, 2,4-dimethoxy-4'-hydroxy- and 4'-allyloxychalcones exhibit potent antimalarial activity in low doses. It is contemplated that this applies generally to the classes to which these chalcones belong, that is, the classes of chalcones defined in claims 21, 24, 25, 26, 29, 32, 35, 38, 40, 41and 46 and that doses of at the most 20 mg per kg body weight per day, such as at the most 10 mg per kg body weight per day or even at the most 5 mg per kg body weight per day, will be preferred dosages for such compounds.

3. When these compounds are used for the treatment of an established infection, they are able to decrease the parasitemia and in most cases clear the infection. This is important because they allow the mice to establish an immune response which then may be able to eventually eliminate the parasites.

EXAMPLE 8

Effect of licochalcone A on *Legionella* and some other bacterial species

Materials and methods:

Drug. Licochalcone A was isolated as described in Example 1.

Bacteria. 20 *Legionella* strains: Five clinical isolates from bronchial secretions and a lung abscess: 2 *Legionella pneumophila* serogroup 1 and 3 *Legionella micdadei* (*L. detroit, L. bari, L. F 1433*). Eight *Legionella pneumophila* serogroups 1-7 and one strain of each of *L. bozemanii, L. dumoffii, L. gormanii, L. micdadei, L. feelei, L. wadsworthii, L. longbeacheae. Staphylococcus aureus* ATCC 25923 was the control strain. The *Legionella* strains were subcultured on buffered charcoal yeast extract with alfa-ketoglutarate (BCYE-α), and the rest of the strains were subcultured on 10% horse blood agar for 48 hours and 24 hours, respectively.

Minimal inhibitory concentrations. Macrodilution rows were made with buffered yeast extract with alfa-ketoglutarate (BYE-α) with 2 ml aliquots in vials, containing various concentrations of licochalcone A. Suspensions of Legionella species and the

other pathogens and commensals were made in BYE-α. All the dilution rows were inoculated to give a final concentration of 10^5 CFU/ml. After incubation at 37°C for 2 and 24 hours, respectively, aliquots of 10 µl were taken from all dilution steps and plated onto BCYE-x agar plates (all *Legionella* species) and to 10% horse blood agar (all non-*Legionella* strains). All the BCYE-x plates were incubated for 48 hours in a humid atmosphere at 37°C and read. The inoculated 10% horse blood agar plates were incubated in a normal atmosphere at 37°C for 24 hours and read.

Results

All the clinical *Legionella pneumophilia* isolates were sensitive to licochalcone A, their MIC-values ranging from 1 to 4 µg/ml, whereas *Legionella gormanii* and the 4 *L. micdadei* isolates had MIC-values from 15 to 500 µg/ml. The Gram positive cocci all had MIC's from 4 to 8 mg/ml. One of the corynebacterium species was very sensitive, having MIC of 0.3 µg/ml.

Table 8.1. *Legionella* species susceptibility to licochalcone A in µg/ml.

	No. of strains	MIC
L. pneumophilia serogr. 1	4	1-4
L. pneumophilia serogr. 2-7	6	2-4
L. bozemanii	1	2
L. domoffii	1	2
L. gormanii	1	500
L. micdadei	1	500
L. micdadei (*Detroit*)	1	15
L. micdadei (*Bari*)	1	60
L. micdadei (*F 1433*)	1	60
L. feelei	1	4
L. wadsworthii	1	2
L. longbeacheae	1	1

Conclusion

Licochalcone A exhibited a clear anti-legionella activity at MIC values form 1 µg/ml, in most cases from 1 to 4 µg/ml. The low MIC for *L. pneumophila*, the human pathogen, is promising and therefore licochalcone A can be considered as a potential drug against respiratory infections. The reason that the MIC was very high for the inhibition of *Legionella micdadei* could be that the cell wall of *L. micdadei* is different

from the cell wall of *L. pneumophila* (Hébert et al, 1984) and therefore, that the uptake of licochalcone A in *L. micdadei* is poorer than the uptake in *L. pneumophila* .

EXAMPLE 9

Effect of licochalcone A on *Mycobacteria* species

Materials and methods

63 strains of mycobacteria were used. The bacteria were grown in Dubos broth media before susceptibility testing. Licochalcone A was dissolved in dimethyl sulfoxide (DMSO) and diluted in distilled water to the desired concentration.

Susceptibility testing was performed radiometrically by using a BECTEC 460-TB apparatus in a confined atmosphere (5% CO_2). Bacterial growth was measured as a function of the ability of the bacteria to catabolize ^{14}C-labelled palmitic acid in the BECTEC 7H12B TB medium during growth, which resulted in the release of ^{14}C-labelled CO_2. The growth was expressed as a numerical value called the growth index (GI) which ranged from 1 to 999. The 7H12 vials were inoculated with 0.1 ml of an appropriately diluted Dubos broth culture to give a final inoculum of about $5x10^4$ colony-forming units (CFU) per ml together with 0.1 ml of different concentrations of licochalcone A. The final concentrations of licochalcone A tested ranged from 1.25 µg/ml to 80 µg/ml. A vial without licochalcone A, but with an inoculum diluted 1:100, was included as a control. The final inoculum was determinated by culturing 0.1 ml from the control vial onto one Lowenstein-Jensen slant. The vials were incubated under stationary conditions at 35°C and growth was monitored by daily GI determination for 7 days. At day 7, 0.1 ml from each vial with a GI reading < 30 was cultured onto one Lowenstein-Jensen slant. Colony counts were enumerated after incubation at 35°C for 3 weeks.

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of licochalcone A which could inhibit 99% or more of the mycobacteria population. Minimal bactericidal concentration (MBC) was defined as the lowest concentration of licochalcone A which killed 99% or more of the mycobacteria population.

Results

Table 9.1. Eighteen different species of *Mycobacteria* were screened for susceptibility of 20 µg/ml of licochalcone A.

Species	
MIC≤20 µg/ml	MIC>20 µg/ml
M. tuberculosis	*M. szulgai*
M. bovis	*M. avium/intracellular*
BCG	*M. scrofulaceum*
M. kansasii	*M. malmoense*
M. xenophii	*M. terrae/triviale*
M. marinum	*M. nonchromogenicum*
	M. smegmatis
	M. flavescens
	M. fortuitum
	M. chelonae

Determinations of MIC and MBC of licochalcone A against strains belonging to the *M. tuberculosis* complex:

M. tuberculosis $mean_{MIC}$ = 7.1 µg/ml
$range_{MIC}$ = 5-10 µg/ml (n=19)
$mean_{MBC}$ = 40 µg/ml (n=2)

M. bovis $mean_{MIC}$ = 15.7 µg/ml
$range_{MIC}$ = 10-20 µg/ml (n=8)

BCG $mean_{MIC}$ = 8.6 µg/ml
$range_{MIC}$ = 5-10 µg/ml (n=3)
$mean_{MBC}$ = 40 µg/ml (n=3)

Determinations of MIC of licochalcone A against strains of *M. avium/intracellular*:

M. avium (AIDS patients): $mean_{MIC}$>80 µg/ml (n=4)
M. avium (non AIDS patients): $mean_{MIC}$>80 µg/ml (n=7)
M. intracellular: $mean_{MIC}$ = 50.0 µg/ml
$range_{MIC}$ = 20-80 µg/ml (n=9)

Table 9.2. Influence of 10% serum on MIC determination of licochalcone A.

MIC (µg/ml) with and without 10% Human Serum: Strain=H37RV

	Ethambutol (40% protein binding)	Ofloxacin (5% protein binding)	Fusidic acid (90% protein binding)	Licochalcone A
MIC_{-serum}	1	0.5	8	5
MIC_{+serum}	2	0.5	32	40

Conclusion

With a proposed "cut off" concentration value of 20 µg/ml, most strains belonging to the *M. tuberculosis* complex were susceptible. The bactericidal concentration was 4 to 8 times the inhibitory concentration which in all strain tested was higher than the "cut off" concentration. All *M. avium/intracellular* strains were on the other hand resistant with MIC ≥20 µg/ml and most with MIC >80 µg/ml. From Table 9.2 it is seen that MIC of licochalcone A increases 8-fold when supplemented with 10% of serum which may indicate that licochalcone A is highly protein-bound.

EXAMPLE 10

Anticoccidial activity of licochalcone A in chickens

The experiment was carried out in collaboration with Korn og Foderstof Kompagniet (KFK) at KFK's Experimental Station (Forsøgsgård, Sdr. Forumvej 18, DK-6715 Esbjerg, Denmark). Licochalcone A was mixed manually with chicken feed one week before use. 2.6 g licochalcone A was mixed with 1 kg rye flour. The mixture was then mixed with ten kg chicken feed.The prepared feed as well as a standard feed used, containing 70 ppm salinomycin which is a known coccidiostatic agent, were stored at a temperature between 10°C and 15°C prior to use.

Parasite strain. *Eimeria tenella* sporulated oocysts were obtained from the Agricultural and Food Council Institute for Animal Health, Compton Laboratory, Berkshire, England. The oocysts were washed and resuspended in 30 ml saline to give a concentration of 15 x 10^6/30 ml. A volume of 0.1 ml (50,000 oocysts) was given to each chicken.

Anticoccidial testing. The experimental set-up consisted of 4 groups of 14-days old

chickens. During the first 14 days of life, all the chickens received chicken feed containing no coccidiostatic agents. The first 3 groups were given 50,000 *E. tenella* oocysts per chicken by oral administration on day 14. Feeding the chickens with the feed preparations described above started one day before infection with the parasite (day 13). The treatment continued for 14 days according to the set-up shown in Table 10.1.

Table 10.1. Experimental set-up for testing anticoccidial activity of licochalcone A.

Groups	Number	Infection with *E. tenella*	Treatment
1	35	Yes	Standard KFK feed containing salinomycin
2	20	Yes	Licochalcone A (10 mg/kg chicken body weight/day)
3	35	Yes	None
4	35	Yes	None

The following parameters were examined and the samples were obtained:

1. All the chickens were weighed once a week as a standard procedure.

2. Mortality of the chickens was observed and recorded on a daily basis.

3. At the end of the experiment (14 days treatment, 28 days old chickens), the chickens were slaughtered and a necropsy was performed for identification of gross pathology. Histopathology was registered in standard HE sections of 10-15 mm of one cecal sac, one transverse and one longitudinal. The sections from each chicken were examined. The pathology was registered according to J. Johnson and W. M. Reid, Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments with chickens. *Experimental Parasitology* **28** (1970), 30-36.

4. Parasite load in the intestine or number per smear may be determined at the end of the experiment. The number of oocysts in 10 viewfields may be counted at 100 x enlargement and the average of 10 fields were used. Oocysts index may calculated as:

$$\frac{\text{Oocysts in infected animal/field} \times 100}{\text{Oocysts in control animal/field}}$$

and may be recorded as follows:

0	= no oocysts
+	= 1 oocysts/field
++	= 1-10 oocysts/field
+++	= >10 oocysts/field

Table 10.2. Effect of licochalcone A on weight gain of chickens. The groups are as shown in Table 10.1. The weights are given in grams per chicken.

Group	Weight	Before treatment	After initiation of treatment	
	7 days old	Weight gain in 7 days	Weight gain in 7 days	Weight gain in 14 days
1	148	215	425	900[a]
2	135	201	414	864[b]
3	138	200	367	803
4	130	204	404	866

[a] Difference between groups 1 (standard feed) and 5 (control infected) after treatment for 14 days is 12%.

[b] Difference between groups 4 (licochalcone A) and 5 (control infected) after treatment for 14 days is 7.6%.

Normal variation in such experiments is ±2%.

Table 10.3. Effect of licochalcone A on feed consumption and mortality of chickens. The groups are as shown in Table 10.1.

Group	Before treatment	After initiation of treatment		Mortality
	after 14 days	after 7 days	after 14 days	
1	1.10	1.30	1.44	0
2	1.15	1.36	1.48	1
3	1.11	1.32	1.46	0

Table 10.4. Effect of licochalcone A on gross lesions induced by *E. tenella* infection. The pathological scores are according to Johnson and Reid and are given as number of chickens with + to ++++ gross pathology/total number in each group. Total percentages with pathological changes are given in the last column.

Group	Pathological scores			% chicken
	Total	Score	Numbers	
1	0/20	0	20	0%
		+	0	
		++	0	
		+++	0	
2	7/20	0	13	35%
		+	6	
		++	0	
		+++	1	
		++++	0	
3	35/40	0	5	87.5%
		+	24	
		++	5	
		+++	4	
		++++	2	
4	0/40	0	40	0%
		+	0	
		++	0	
		+++	0	
		++++	0	

0 = normal
\+ = few hemorrhages (punctuate)
++ = blood in lumen, mucosal lesions, thickened walls
+++ = blood in lumen (coagulated, clumps), detached epithelium
++++ = diffuse bleeding, obstructed cecum, big masses mixed with lots of oocysts

Conclusions

The results from this experiment clearly indicate that licochalcone A is able to control *E. tenella* infection in chickens. This is documented by the following:

1. No mortality was observed in the group receiving licochalcone A (Table 10.3).

2. A 7.6% increase in weight gain in chickens receiving licochalcone A as compared to the infected group not receiving any cocciostatic treatment (Table 10.2). The normal variation in such experiments is ±2%.

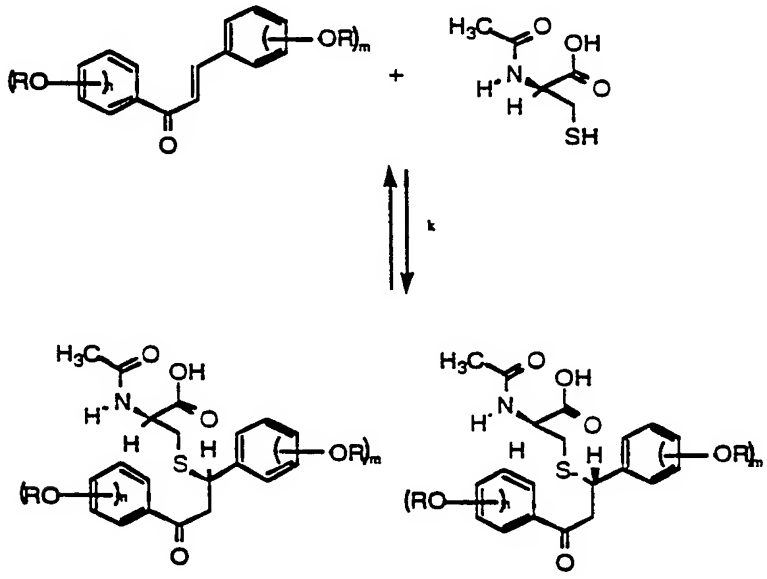
3. Feed consumption, measured as the amount of feed consumed per kg chicken weight gain in the group receiving licochalcone A was the lowest among all the 6 groups both on 7 days of treatment and on 14 days of treatment (Table 10.3). The feed consumption of the group receiving licochalcone A was even lower than the group receiving standard chicken feed containing salinomycin which is a know coccidiostatic agent. This indicates that licochalcone A might have a growth promoting effect or another form of nutritional value.

4. The percentages of chickens showing pathological signs were much lower in the group receiving licochalcone A than in the infected control group (Table 10.4).

The chickens receiving licochalcone A did not perform the same way as those receiving standard chicken feed containing salinomycin. However, when comparing the licochalcone A group with the group receiving standard feed, it should be noted that this group received standard feed which besides a coccidiostatic agent also contains larger amounts of nutrients, vitamin, and growth promoting factors. In the above experiment, licochalcone A did not show a complete protection against *E. tenella* infection. This is probably due to the dosage of licochalcone A used in the experiment. It should also be mentioned that the experimental infection is a much stronger form of infection than the infection which will normally be encountered in practice.

EXAMPLE 11

Estimation of the rate of the reaction between N-acetyl-L-cysteine and chalcones



150 µg of the chalcone in question and 10 mg (0.06 mmol) of N-acetyl-L-cysteine were dissolved in 2.5 ml of methanol-water-potassium phosphate buffer (40:10:50 v/v, pH 7.5), and the solution was left at 30°C. The decline of the chalcone concentration was followed by HPLC. The following experimental setups were used:

1. Column Spherisorb ODS-2 (120 x 4.6 m, 5 µm), eluent acetonitrile-aqueous acetic acid (2%) in ratios 43:57 or 50:50, flow rate 1.5 ml/min, detection at 254 and 360 nm. The polar eluent was used for the following chalcones: licochalcone A, chalcone, 2,4-dimetoxychalcone and 4,4'-dihydroxychalcone. The apolar eluent was used for the following chalcones: 3,4,5-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone, 2,4-dimethoxy-4'-(3-methylbut-2-enyloxy)chalcone and 2,4,6-trimethoxy-4'-(3-methylbut-2-enyloxy)-chalcone.

2. Column Polygosil Si 60 (120 x 4.6 mm, 5 µm), eluent methanol-water-0.2 M potassium phosphate buffer (65:30:5, pH 7.5) added cetyltrimethylammonium bromide to a final concentration of 2.5 mM, column temperature 45°C, flow rate 1.0 ml/min, detection at 254 nm. This system was used i the case of 4'-methoxychalcone.

Results

The rate of decline of the chalcone concentration was followed by HPLC. The rate constant was estimated using Grafit and assuming that the reaction followed first order kinetic; i.e.:

$$[c] = [c_0]\exp(-kt)$$

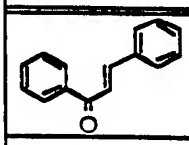
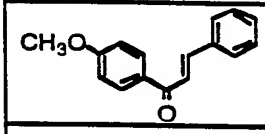
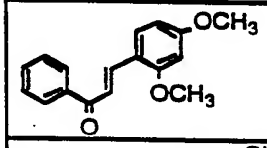
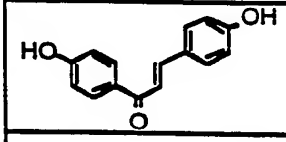
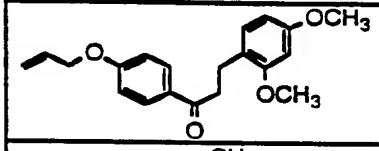
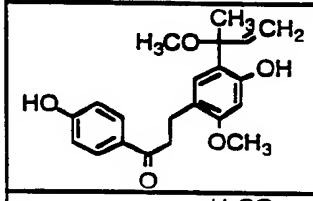
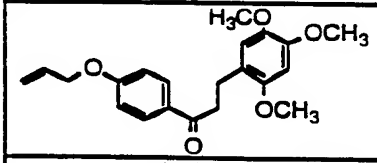
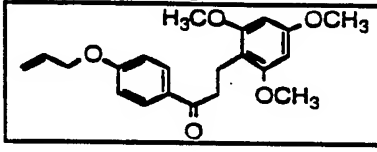
where [c] is the concentration of the chalcone in question at time t, $[c_0]$ is the concentration of the chalcone in question at time zero, k is the rate constant, and t the time. In all cases, very good fits between the observed concentrations and the concentrations calculated using the estimated rate constants were obtained.

Conclusion

Introduction of oxygen functions in the 2-position, the 4-position or the 2- and the 4-positions, or in the 4'-position appears to decrease the reaction rate. In contrast, comparison of the rate constants of 3,4,5-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone and 2,4,6-trimethoxy-4'-(3-methylbut-2-enyloxy)chalcone indicates that introduction of oxygen functions in the 3- and 5-position increases the electron density at the double bond and consequently reduces the reactivity toward nucleophilic reagents, whereas the inductive effects of oxygen in the 3- or 5-position will decrease the

electron density at the double bond. Analogously, the Hammet σ_P constant for methoxy is -0.27 but σ_m is 0.12.

Table 11.1 Estimated rate constants.

Formula	Number of oxygens	k (min^{-1})
	0	0.034
	2	0.016
	2	0.0033
	2	0.0020
	3	0.0011
	3	0.00058
	4	0.0175
	4	0.0006

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CLAIMS

1. The use of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed, food or drinking water for the treatment or prophylaxis of a disease caused by a microorganism or a parasite in an animal, including a vertebrate, such as a bird, a fish or a mammal, including a human,

the microorganism or parasite being selected from

parasitic protozoa, in particular tissue and blood protozoa such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium, Pneumocystis, Babesia* and *Theileria;* intestinal protozoan flagellates such as *Trichomonas* and *Giardia;* intestinal protozoan *Coccidia* such as *Eimeria, Isospora, Cryptosporidium; Cappilaria, Microsporidium, Sarcocystis, Trichodina, Trichodinella, Dacthylogurus, Pseudodactylogurus, Acantocephalus, Ichthyophtherius, Botrecephalus;* and intracellular bacteria, in particular *Mycobacterium, Legionella* species, *Listeria,* and *Salmonella.*

2. The use according to claim 1, wherein the aromatic compound, in a concentration in which it causes less than 50% reduction of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using phytohemagglutinin (PHA), meets at least one of the following criteria:

a) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine,

b) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Plasmodium falciparum* by at least 80%, as determined by uptake of tritiated hypoxanthine,

c) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Eimeria tenella* in chicken fibroblast cell cultures by at least 70% ,as determined by counting the parasites,

d) the aromatic compound is capable of inhibiting *in vitro* the growth or multiplication of *Mycobacterium tuberculosis* or *Legionella pneumophila* by at least 50%, as determined by colony counts.

3. The use according to claim 2, wherein the aromatic compound, in a concentration in which it causes less than 40% reduction, preferably less than 30% reduction, more preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA, meets at least one of the criteria a) to d).

4. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Leishmania major* promastigotes by at least 80%, as determined by uptake of tritiated thymidine, in a concentration of the compound in which it causes less than 50% reduction, preferably less than 40% reduction, more preferably less than 30% reduction, most preferably less than 20% reduction, of the thymidine uptake by human lymphocytes in the Lymphocyte Proliferation Assay using PHA.

5. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 5 herein in a dose of up to 20 mg per kg body weight, preferably in a dose of up to 10 mg per kg body weight, once daily for 40 days to female BALB/c mice which have been infected with *L. major* ((10^7/mouse), the administration being initiated one week after infection, is capable of preventing increase in lesion size by at least 60%, preferably at least 80%, more preferably at least 90%.

6. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Leishmania* in humans or dogs, and the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 6 herein in a dose of up to 20 mg per kg body weight , preferably in a dose of up to 10 mg per kg body weight, two times daily for 7 days to male Syrian golden hamsters which have been infected with *L. donovani* promastigotes (2 x 10^7/hamster), the administration being initiated one day after infection, is capable of reducing the parasite load in the liver of the hamsters by at least 60%, preferably by at least 80%, and more preferably by at least 90%.

7. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of malaria caused by *Plasmodium spp.* in humans, and the aromatic compound is capable of inhibiting *in vitro* the growth of *Plasmodium falciparum* by at least 80%, as measured by uptake of tritiated hypoxanthine, in a concentration of the compound in which it causes less than 50%

reduction, preferably less than 40% reduction, more preferably less than 20% reduction, of the thymidine uptake by human lymphocytes, as measured by the Lymphocyte Proliferation Assay using PHA.

8. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by *Plasmodium spp.* in humans, and the aromatic compound, when administered intraperitoneally in the *in vivo* test described in Example 7 herein in a dose of up to 20 mg per kg body weight two times daily for 6 days to female BALB/c mice which have been infected with malaria *P. yoelii* (2×10^5/mouse), the administration being initiated one day after infection, is able to prevent increase in the parasitemia during the administration period.

9. The use according to claim 8, wherein the aromatic compound, or the prodrug, when administered intraperitoneally in the *in vivo* test described in Example 7 herein in a dose of up to 20 mg per kg body weight, preferably in a dose of up to 5 mg per kg body weight, most preferably in a dose of up to 2.5 mg per kg body weight, two times daily for 8 days to 8 weeks old female BALB/c mice which have been infected with malaria *P. yoelii* strain YM (1×10^6/mouse), the administration being initiated one day after infection, is capable of clearing the parasite from the mice within at the most 23 days, preferably within at the most 17 days.

10. The use according to claim 1 of an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, for the preparation of a pharmaceutical composition or a medicated feed or drinking water for the treatment or prophylaxis of diseases caused by *Coccidia* in poultry such as chickens or turkeys, wherein the aromatic compound, or the prodrug, when administered to chickens with the feed in a concentration of up to 400 ppm, preferably in a concentration of up to 120 ppm, more preferably in a concentration of up to 60 ppm, for at most 28 days in the *in vivo* test described in Example 10 herein, is capable of controlling infection by *Eimeria tenella* in at least 60%, preferably at least 80%, of the chickens and preventing pathological alterations in at least 50%, preferably in at least 65%, of the chickens.

11. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Mycobacteria* in humans or animals such as cattle, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Mycobacteria tuberculosis in vitro* in the test described in Example 9 herein at a mean MIC of up to 20 µg per ml, preferably up to 10 µg per ml, more preferably up to 5 µg

per ml, and, in the same concentration, causes less than 50% reduction, preferably less than 40% reduction, more preferably less than 20% reduction, of the thymidine uptake of human lymphocytes as measured by The Lymphocyte Proliferation Assay.

12. The use according to claim 1, wherein the pharmaceutical composition is a composition for the treatment or prophylaxis of diseases caused by intracellular bacteria such as *Legionella* in humans, and the aromatic compound is one which is capable of inhibiting the growth and multiplication of *Legionella pneumophila in vitro* in the test described in Example 8 herein at a mean MIC of up to 20 µg per ml, preferably up to 10 µg per ml, more preferably up to 5 µg per ml, and, in the same concentration, causes less than 50% reduction, preferably less than 40% reduction, more preferably less than 20% reduction, of the thymidine uptake of human lymphocytes as measured by The Lymphocyte Proliferation Assay.

13. The use according to any of claims 1-3, wherein the disease is human leishmaniasis caused by *Leishmania donovani, L. infantum, L. aethiopica, L. major, L. tropica, L. mexicana complex*, or *L. braziliensis complex* or human malaria caused by *Plasmodium falciparum, P. ovale, P. vivax*, or *P. malariae*.

14. The use according to any of claims 1-3, wherein the disease is a parasitic disease in livestock, such as *Babesia* in cattle, or a parasitic disease in birds, such as a disease caused by *Coccidia* such as *Eimeria tenella* in poultry such as chicken or turkey, or a parasitic disease in fish, such as *Pseudodactylogurus* or *Trichodina*.

15. The use according to any of the preceding claims, wherein the aromatic compound is a bis-aromatic α,β-unsaturated ketone of the general formula I

$$X_m\text{-}Ar^1\text{-}CO\text{-}W\text{-}Ar^2\text{-}Y_n \qquad I$$

wherein

W is either -CR=CR- or -C≡C-, wherein each R independently of the other R designates hydrogen, $C_{1\text{-}3}$ alkyl, or halogen,

Ar^1 and Ar^2 are the same or different and each designate an aromate selected from phenyl and 5- or 6-membered unsaturated heterocyclic rings containing one, two or three heteroatoms selected from oxygen, sulphur, and nitrogen, such as furanyl, thiophenyl, pyrrolyl, imidazolyl, isoxazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, or pyrimidinyl, which aromate may be substituted with one or more substituents selected from

halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups;

Y and X are the same or different and each designate a group AR_H or a group AZ, wherein A is -O-, -S-, -NH-, or -N(C_{1-6} alkyl)-, R_H designates C_{1-6} straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, and Z designates H or (when the compound is a prodrug) a masking group which is readily decomposed under conditions prevailing in the animal body to liberate a group AH, in which A is as defined above; m designates 0, 1 or 2, and n designates 0, 1, 2 or 3, whereby, when m is 2, then the two groups X are the same or different, and when n is 2 or 3, then the two or three groups Y are the same or different, with the proviso that n and m are not both 0.

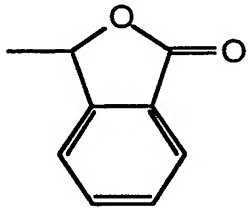
16. The use according to claim 15, wherein Z, when designating a masking group, is selected from the below groups (A)-(E)

-CO-R' (A)

$-CON(CH_3)_2$ (B)

-CR*R**-O-R'' (C)

-CR*R**-O-CO-R''' (D)



(E)

wherein R* and R** each independently designate hydrogen or C_{1-3} alkyl, R', R'' and R''' each designate C_{1-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 15.

17. The use according to claims 15 or 16, wherein Ar^1, or Ar^2 or both independently are phenyl or an aromatic 5- or 6-membered heterocyclic ring containing one, two or three heteroatoms selected from oxygen, sulphur and nitrogen, n is 0, 1, 2, or 3, m is 0, 1 or 2, at least one of the groups X is in a position in Ar^1 most remote relative to and/or next to the position through which Ar^1 is bound to the carbonyl group, and at least one of the groups Y is in a position in Ar^2 most remote relative to and/or next to

the position through which Ar^2 is bound to W.

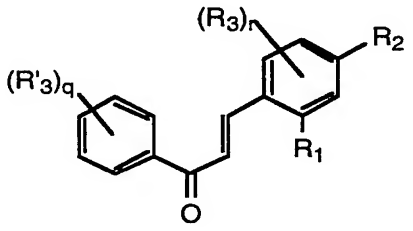
18. The use according to any of claims 15-17, in which the compound of formula I is a compound of formula II

$$X_m\text{-Ph-C(O)-CH=CH-Ph-}Y_n \qquad \text{II}$$

wherein Ph designates phenyl, and X_m and Y_n are as defined in claim 15, and each phenyl group may be substituted with one or more substituents selected from halogen; nitro; nitroso; and C_{1-12}, preferably C_{1-6}, straight or branched aliphatic hydrocarbyl which may be saturated or may contain one or more unsaturated bonds selected from double bonds and triple bonds, which hydrocarbyl may be substituted with one or more substituents selected from hydroxy, halogen, amino, and amino which is optionally alkylated with one or two C_{1-6} alkyl groups.

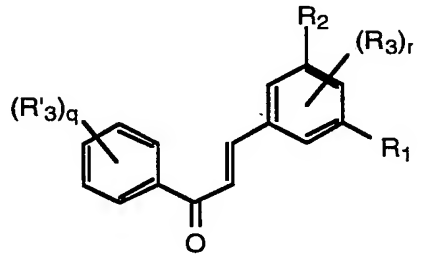
19. The use according to claim 18, in which X and/or Y is OH or a group OR_H, in which R_H is as defined in claim 15, or OZ, in which Z is a masking group which is readily decomposed under conditions prevailing in the animal body to liberate the group OH, in particular one of the groups (A)-(E) as defined in claim 16, preferably pivaloyl, pivaloyloxymethyl or N,N-dimethylcarbonyl.

20. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



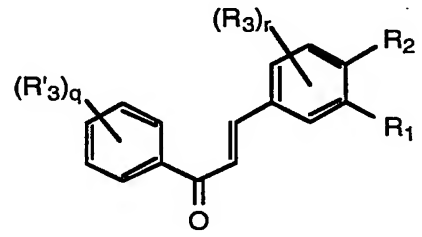
wherein R_1 and R_2 independently designate $A(W^*)_p$, wherein W^* is H, R_H or a masking group Z as defined in claim 15 or 16, and A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2; and R_3' designate H or $A(W^*)_p$, wherein W^* designates H, R_H or a masking group Z as defined in claim 15 or 16, q is an integer from 1 to 5, r is 1 or 2, and A designates S, N or O, whereby, when A designates S or O, then p designates 1, and when A designates N, then p designates 2, and R_3 designates H or R_H as defined in claim 15.

21. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



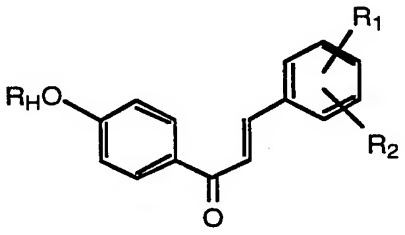
wherein R_1, R_2, R_3, R'_3, q and r are as defined in claim 20.

22. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



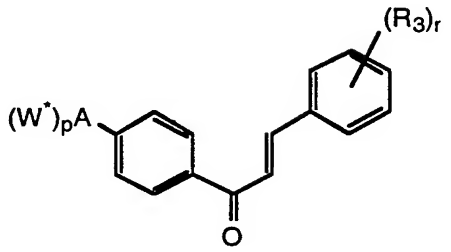
wherein R_1, R_2, R_3, R'_3, q and r are as defined in claim 20.

23. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



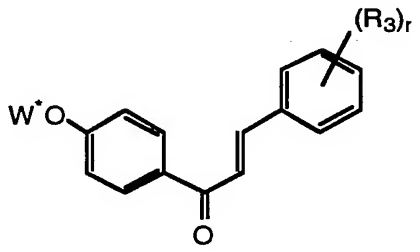
wherein R_1 and R_2 are as defined in claim 20, preferably hydroxy or lower alkoxy such as methoxy or ethoxy, and R_H is as defined in claim 15, R_H preferably being prop-2-enyloxy.

24. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



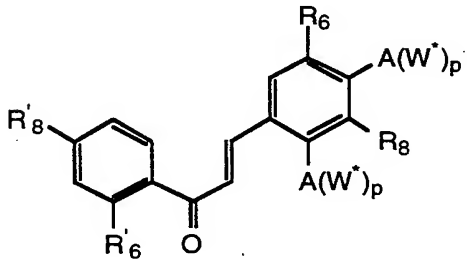
wherein R_3 and r are as defined in claim 20, and W* designates H, R_H or a masking group A as defined in claim 15 or 16.

25. The use according to claim 24, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R_3, r and W* are as defined in claim 24.

26.The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula

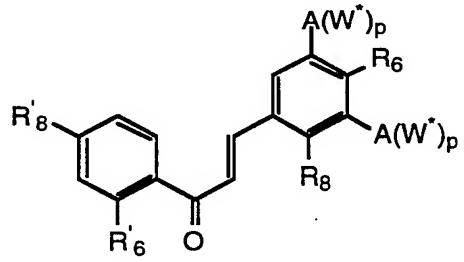


wherein R_6 and R_8 designate H or R_H as defined in claim 15, A and W* are as defined in claim 20 and R'_6 and R'_8 each designate H or $A(W^*)_p$ as defined in claim 20,

with the note that 2,4-dimethoxy-4'-(N,N-dimethylcarbamoyloxy)chalcone, 2,4-

dimethoxy-4'-hydroxychalcone, 2,4-dimethoxy-4'-methoxymethoxychalcone, 2,4-dimethoxy-4'-allyl-oxychalcone, 4'-(2-methoxyethoxymethoxy)-4-(3-methylbut-2-enyloxy)-2-methoxychal-cone, 2,4-dimethoxy-2'-(3-methyl-2-enyloxy)chalcone, 2,4-dimethoxychalcone, and 2,4-dimethoxy-4'-pivaloyloxymethoxychalcone are mentioned in PCT/DK93/00088.

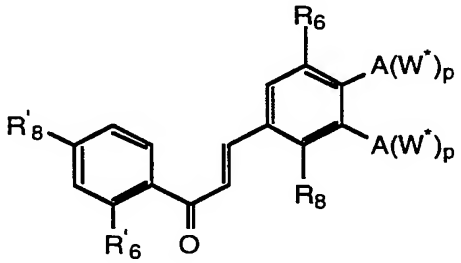
27. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R_6, R_8, R'_6, R'_8 and $A(W^*)_p$ are as defined in claim 26,

with the note that 3,5-dimethoxy-4'-prop-2-enyloxychalcone is ment ioned in PCT/DK93/00088.

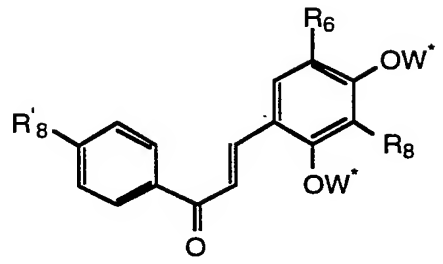
28. The use according to claim 18, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R'_6, R'_8, R_6, R_8, A and W^* are as defined in claim 26,

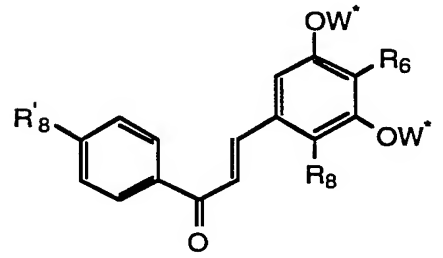
with the exception of 3,4-dimethoxy-4'-hydroxychalcone, 3,4-dihydroxy-2',4'-dihydroxychalcone, 3,4-dimethoxy-2',4'-dihydroxychalcone and 4-hydroxy-3-methoxy-2',4'-dihydroxychalcone, and with the note that 3,4-dimethoxy-4'-prop-2-enyloxychalcone is mentioned in PCT/DK93/00088.

29.The use according to claim 26, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



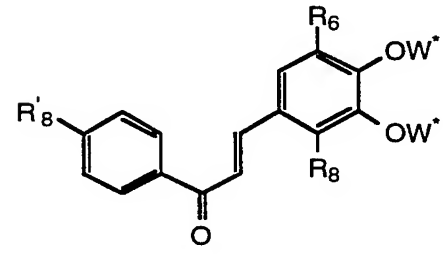
wherein R'_8, R_6, R_8 and W* are as defined in claim 26, in particular when the bis-aromatic α,β-unsaturated ketone is a compound as defined in claim 41 or 42, or licochalcone A, licochalcone C, 2,4,4'-trihydroxychalcone, 2, 4'-dimethoxy-4'-hydroxychalcone and 2,4,4'-trimethoxychalcone.

30.The use according to claim 27, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R'_8, R_6, R_8 and W* are as defined in claim 26, in particular when the bis-aromatic α,β-unsaturated ketone is a compound as defined in claim 46.

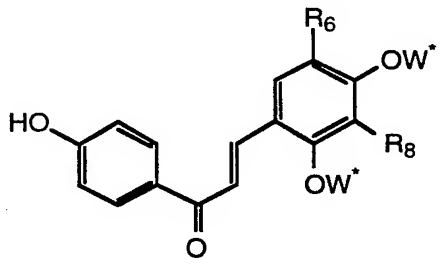
31.The use according to claim 28, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R'_8, R_6, R_8 and W* are as defined in claim 26, in particular when the bis-

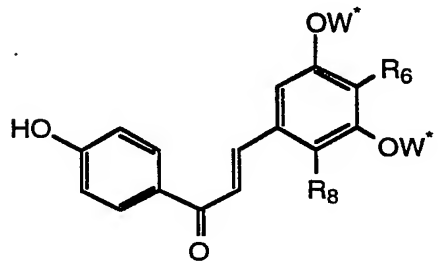
aromatic α,β-unsaturated ketone is a compound as defined in claim 44, or 3,4-dimethoxy-4'-hydroxychalcone.

32.The use according to claim 29, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



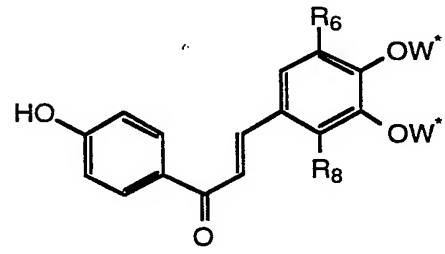
wherein R_6, R_8 and W* are as defined in claim 26.

33.The use according to claim 30, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



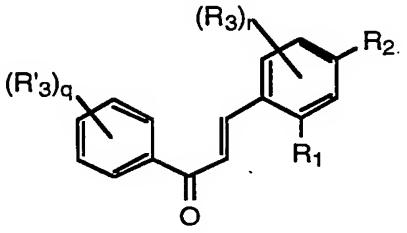
wherein R_6, R_8 and W* are as defined in claim 26.

34.The use according to claim 31, wherein the bis-aromatic α,β-unsaturated ketone has the general formula



wherein R_6, R_8 and W* are as defined in claim 26.

35. Novel bis-aromatic α,β-unsaturated ketones of the general formula

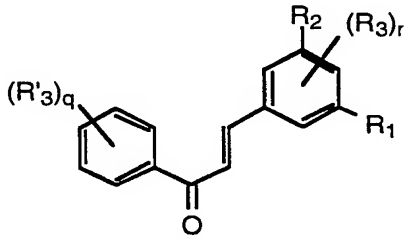


wherein R_1, R_2, R_3, R'_3, q and r are as defined in claim 20, including 6-methyl-2-methoxy-4,4'-dihydroxychalcone,

with the proviso that, when R_3 is H, then the group consisting of R_1, R_2 and $(R'_3)_q$ is different from any combination comprising three hydroxy groups, three methoxy groups; one methoxy group and two hydroxy groups; two methoxy groups and one hydroxy group; one methoxy group and two hydroxy groups; except that the compound 2,4-dimethoxy-4'-hydroxychalcone is **not** exempted from the scope of the present claim,

and with the exception of licochalcone A, licochalcone C, 3-[4-hydroxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[5-(1,1-dimethylprop-2-enyl)-2,4-dimethoxyphenyl]-1-[4-(methoxy)-phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-(4-acetyloxyphenyl)-2-prop-1-one, 3-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-1-[4-[(3,7,11-trimethyl-2,6-dodecatri-10-enyl)oxy]phenyl]-2-prop-1-one, 2,4-dihydroxy-3-methylchalcone and 1-[4-(methoxymethoxy)phenyl]-3-[2-methoxy-4-methyl-2-butenyloxy]-2-propenone-1, and with the note that 2,4-dimethoxy-4'-(N,N-dimethylcarbamoyloxy)-chalcone, 2,4-dimethoxy-4'-hydroxychalcone, 2,4-dimethoxy-4'-methoxymethoxy-chalcone, 2,4-dimethoxy-4'-prop-2-enyloxychalcone, 4'-(2-methoxyethoxymethoxy)-4-(3-methylbut-2-enyloxy)-2-methoxychalcone, 2,4-dimethoxy-2'-(3-methylbut-2-enyloxy)-chalcone, 2,4-dimethoxychalcone and 2,4-dimethoxy-4'-pivaloyloxymethoxychalcone are mentioned in PCT/DK93/00088.

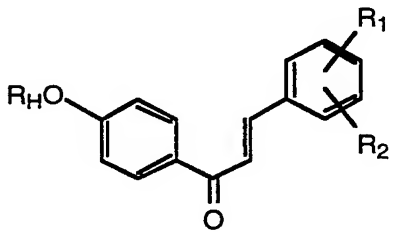
36. Novel bis-aromatic α,β-unsaturated ketones of the general formula



wherein R_1, R_2,R_3, R'_3, q and r are as defined in claim 20,

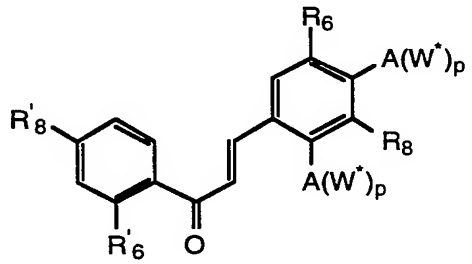
with the note that 3,5-dimethoxy-4'-prop-2-enyloxychalcone is mentioned in PCT/DK93/00088.

37. Novel bis-aromatic α,β-unsaturated ketones the general formula



wherein R_1 and R_2 are as defined in claim 20 preferably hydroxy or lower alkoxy such as methoxy or ethoxy, and R_H is as defined in claim 15, with the proviso that R_H is not methyl.

38. Novel bis-aromatic α,β-unsaturated ketones of the general formula



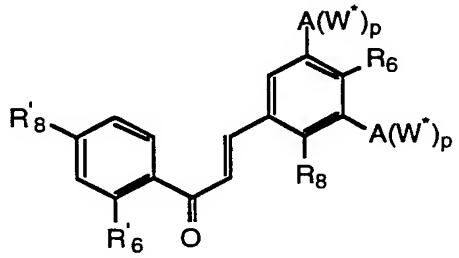
wherein R_6 and R_8, A and W* are as defined in claim 26, R'_6 designates $A(W^*)_p$ and R'_8 designates H, or R'_8 designates $A(W^*)_p$ and R'_6 designates H, or both R'_6 and R'_8 designate H, with the proviso that when R_6 and R_8 both are H, then at least one W* designates a masking group Z as defined in claim 15 or 16, whereby when the masking group is a group -CO-R', then R' is C_{2-6} alkyl or is an aromate Ar^1 or Ar^2 as defined in claim 15, and R_H is as defined in claim 15,

with the proviso that when R_6 and R_8 are H, then the group consisting of the two $A(W^*)_p$-substituents and one of R'_6 and R'_8 is different from any combination comprising three hydroxy groups, three methoxy groups; one methoxy group and two hydroxy groups; two methoxy groups and one hydroxy group; one methoxy group and two hydroxy groups; except that the compound 2,4-dimethoxy-4'-hydroxychalcone is

not exempted from the scope of the present claim,

with the exception of licochalcone A, licochalcone C, 3-[4-hydroxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-[4-(methoxymethoxy)phenyl]-2-propen-1-one, 3-[5-(1,1-dimethylprop-2-enyl)-2,4-dimethoxyphenyl]-1-[4-(methoxy-)phenyl]-2-propen-1-one, 3-[4-acetyloxy-5-(1,1-dimethylprop-2-enyl)-2-methoxyphenyl]-1-(4-acetyl-oxyphenyl)-2-prop-1-one, 3-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-1-[4-[(3,7,11-trimethyl-2,6-dodecatri-10-enyl)oxy]phenyl]-2-prop-1-one, 2,4-dihydroxy-3-methylchalcone, and 1-[4-(methoxymethoxy)phenyl]-3-[2-methoxy-4-[3-(methyl-2-butenyl)oxy]]-2-propenone-1, 2,4-dihydroxy-3-methylchalcone, and with the note that 2,4-dimethoxy-4'-(N,N-dimethylcarbamoyloxy)chalcone, 2,4-dimethoxy-4'-hydroxy-chalcone, 2,4-dimethoxy-4'-methoxymethoxychalcone, 2,4-dimethoxy-4'-prop-2-enyloxy-chalcone, 4'-(2-methoxyethoxymethoxy)-4-(3-methylbut-2-enyloxy)-2-methoxychalcone, 2,4-dimethoxy-2'-(3-methylbut-2-enyl-oxy)chalcone, 2,4-dimethoxychalcone and 2,4-dimethoxy-4'-pivaloyloxymethoxy-chalcone are mentioned in PCT/DK93/00088.

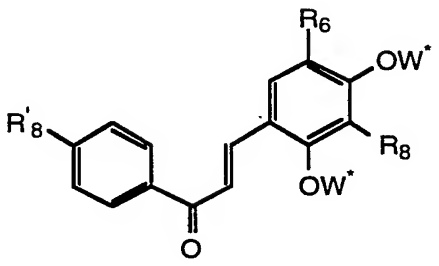
39. Novel bis-aromatic α,β-unsaturated ketones of the general formula



wherein R_6 and R_8 are H or R_H, R'_6 and R'_8 designate H or A(W^*)$_p$, and R_H is as defined in claim 15, and A, W^* and p are as defined in claim 26,

with the note that 3,5-dimethoxy-4'-prop-2-enyloxychalcone is mentioned in PCT/DK93/00088.

40. Novel bis-aromatic α,β-unsaturated ketones according to claim 38 of the general formula



wherein R'_8, R_6, R_8 and W* are as defined in claim 26, subject to the provisos, exceptions and notes of claim 38.

41. Compounds according to claim 40 which are
2,4-dimethoxy-4'-methoxychalcone,
2,4-diethoxy-4'-methoxychalcone,
2,4-di-n-propoxy-4'-methoxychalcone,
2,4-diisopropoxy-4'-methoxychalcone,
2,4-di-n-butoxy-4'-methoxychalcone,
2,4-di-t-butoxy-4'-methoxychalcone,
2,4-dimethoxy-5-methyl-4'-methoxychalcone,
2,4-diethoxy-5-methyl-4'-methoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-methoxychalcone,
2,4-diisopropoxy-5-methyl-4'-methoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-methoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-methoxychalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-methoxychalcone,
2,4-dimethoxy-5-propyl-4'-methoxyhalcone,
2,4-diethoxy-5-propyl-4'-methoxychalcone,
2,4-di-n-propoxy-5-propyl-4'-methoxychalcone,
2,4-diisopropoxy-5-propyl-4'-methoxyhalcone,
2,4-di-n-butoxy-5-propyl-4'-methoxychalcone,
2,4-di-t-butoxy-5-propyl-4'-methoxyhalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,

2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-methoxy-chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-methoxychalcone,

2,4-dimethoxy-4'-ethoxychalcone,
2,4-diethoxy-4'-ethoxychalcone,
2,4-di-n-propoxy-4'-ethoxychalcone,
2,4-diisopropoxy-4'-ethoxychalcone,
2,4-di-n-butoxy-4'-ethoxychalcone,
2,4-di-t-butoxy-4'-ethoxychalcone,
2,4-dimethoxy-5-methyl-4'-ethoxychalcone,
2,4-diethoxy-5-methyl-4'-ethoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-ethoxychalcone,
2,4-diisopropoxy-5-methyl-4'-ethoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-ethoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-ethoxychalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-ethoxychalcone,
2,4-dimethoxy-5-propyl-4'-ethoxyhalcone,
2,4-diethoxy-5-propyl-4'-ethoxychalcone,
2,4-di-n-propoxy-5-propyl-4'-ethoxychalcone,
2,4-diisopropoxy-5-propyl-4'-ethoxyhalcone,
2,4-di-n-butoxy-5-propyl-4'-ethoxychalcone,
2,4-di-t-butoxy-5-propyl-4'-ethoxyhalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,

2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-ethoxychalcone,

2,4-dimethoxy-4'-propoxychalcone,
2,4-diethoxy-4'-propoxychalcone,
2,4-di-n-propoxy-4'-propoxychalcone,
2,4-diisopropoxy-4'-propoxychalcone,
2,4-di-n-butoxy-4'-propoxychalcone,
2,4-di-t-butoxy-4'-propoxychalcone,
2,4-dimethoxy-5-methyl-4'-propoxychalcone,
2,4-diethoxy-5-methyl-4'-propoxychalcone,
2,4-di-n-propoxy-5-methyl-4'-propoxychalcone,
2,4-diisopropoxy-5-methyl-4'-propoxychalcone,
2,4-di-n-butoxy-5-methyl-4'-propoxychalcone,
2,4-di-t-butoxy-5-methyl-4'-propoxychalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-propoxychalcone,
2,4-dimethoxy-5-propyl-4'-propoxyhalcone,
2,4-diethoxy-5-propyl-4'-propoxychalcone,
2,4-di-n-propoxy-5-propyl-4'-propoxychalcone,
2,4-diisopropoxy-5-propyl-4'-propoxyhalcone,
2,4-di-n-butoxy-5-propyl-4'-propoxychalcone,
2,4-di-t-butoxy-5-propyl-4'-propoxyhalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,

2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone, and
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,

2,4-dimethoxy-4'-butyloxychalcone,

2,4-dimethoxy-4'-hexyloxychalcone,

3-methyl-2-methoxy-4,4'-dihydroxychalcone,

5-hexyl-2-methoxy-4,4'-dihydroxychalcone,

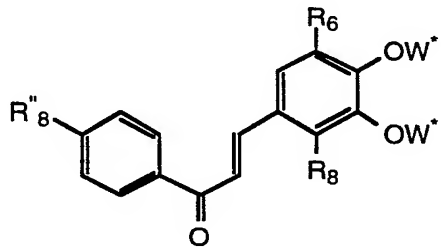
and with the note that they are mentioned in PCT/DK00088, the following compounds:

2,4-diethoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-methyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
2,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

2,4-dimethoxy-4'-hydroxychalcone,
2,4-diethoxy-4'-hydroxychalcone,
2,4-di-n-propoxy-4'-hydroxychalcone,
2,4-diisopropoxy-4'-hydroxychalcone,
2,4-di-n-butoxy-4'-hydroxychalcone,
2,4-di-t-butoxy-4'-hydroxychalcone,
2,4-dimethoxy-5-methyl-4'-hydroxychalcone,
2,4-diethoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-methyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-methyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-methyl-4'-hydroxychalcone,
2,4-di-t-butoxy-5-methyl-4'-hydroxychalcone,
2,4-imethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diethoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-propoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-diisopropoxy-5-prop-2-enyl-4'-hydroxychalcone,
2,4-di-n-butoxy-5-prop-2-enyl-4'-hydroxychalcone, and
2,4-di-t-butoxy-5-prop-2-enyl-4'-hydroxychalcone.

42. Novel bis-aromatic α,β-unsaturated ketones of the general formula



wherein R_6, R_8 and W* are as defined in claim 26, and R''_8 is prop-2-enyloxy or propoxy,

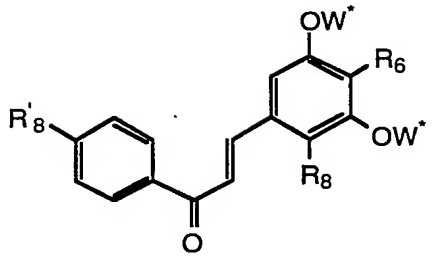
with the note that 3,4-dimethoxy-4'-prop-2-enyloxychalcone is mentioned in PCT/DK93/00088.

43. Compounds according to claim 42 which are
3,4-diethoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-propyl-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone.

3,4-dimethoxy-4'-propoxychalcone,

3,4-diethoxy-4'-propoxychalcone,
3,4-di-n-propoxy-4'-propoxychalcone,
3,4-diisopropoxy-4'-propoxychalcone,
3,4-di-n-butoxy-4'-propoxychalcone,
3,4-di-t-butoxy-4'-propoxychalcone,
3,4-dimethoxy-2-methyl-4'-propoxychalcone,
3,4-diethoxy-2-methyl-4'-propoxychalcone,
3,4-di-n-propoxy-2-methyl-4'-propoxychalcone,
3,4-diisopropoxy-2-methyl-4'-propoxychalcone,
3,4-di-n-butoxy-2-methyl-4'-propoxychalcone,
3,4-di-t-butoxy-2-methyl-4'-propoxychalcone,
3,4-dimethoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-diethoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-n-propoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-diisopropoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-n-butoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-di-t-butoxy-5-prop-2-enyl-4'-propoxychalcone,
3,4-dimethoxy-5-propyl-4'-propoxychalcone,
3,4-diethoxy-5-propyl-4'-propoxychalcone,
3,4-di-n-propoxy-5-propyl-4'-propoxychalcone,
3,4-diisopropoxy-5-propyl-4'-propoxychalcone,
3,4-di-n-butoxy-5-propyl-4'-propoxychalcone,
3,4-di-t-butoxy-5-propyl-4'-propoxychalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-dimethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diethoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-propoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-diisopropoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone,
3,4-di-n-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone, and
3,4-di-t-butoxy-5-(1,1-dimethylethyl)-4'-propoxychalcone.

44. Novel bis-aromatic α,β-unsaturated ketones according to claim 39 of the general formula



wherein R'_8, R_6, R_8 and W* are as defined in claim 26,

with the note that 3,5-dimethoxy-4'-prop-2-enyloxychalcone is mentioned in PCT/DK93/00088.

45. Compounds according to claim 44 which are
3,5-diethoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4'-(prop-2-enyloxy)chalcone,

3,5-dimethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-propyl-4'-(prop-2-enyloxy)chalcone,

3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

3,5-dimethoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-methyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-prop-2-enyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-propyl-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-(prop-2-enyloxy)chalcone,

3,5-dimethoxy-2-methyl-4'-methoxychalcone,
3,5-diethoxy-2-methyl-4'-methoxychalcone,
3,5-di-n-propoxy-2-methyl-4'-methoxychalcone,
3,5-diisopropoxy-2-methyl-4'-methoxychalcone,
3,5-di-n-butoxy-2-methyl-4'-methoxychalcone,
3,5-di-t-butoxy-2-methyl-4'-methoxychalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-diethoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-methoxychalcone,
3,5-dimethoxy-2-propyl-4'-methoxychalcone,
3,5-diethoxy-2-propyl-4'-methoxychalcone,
3,5-di-n-propoxy-2-propyl-4'-methoxychalcone,
3,5-diisopropoxy-2-propyl-4'-methoxychalcone,
3,5-di-n-butoxy-2-propyl-4'-methoxychalcone,
3,5-di-t-butoxy-2-propyl-4'-methoxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-methoxychalcone,

3,5-dimethoxy-2-methyl-4'-methoxychalcone,
3,5-diethoxy-4-methyl-4'-methoxychalcone,
3,5-di-n-propoxy-4-methyl-4'-methoxychalcone,
3,5-diisopropoxy-4-methyl-4'-methoxychalcone,
3,5-di-n-butoxy-4-methyl-4'-methoxychalcone,
3,5-di-t-butoxy-4-methyl-4'-methoxychalcone,
3,5-dimethoxy-4-prop-2-enyl-4'-methoxychalcone,

3,5-diethoxy-4-prop-2-enyl-4'-methoxychalcone,
3,5-di-n-propoxy-4-prop-2-enyl-4'-methoxychalcone,
3,5-diisopropoxy-4-prop-2-enyl-4'-methoxychalcone,
3,5-di-n-butoxy-4-prop-2-enyl-4'-methoxychalcone,
3,5-di-t-butoxy-4-prop-2-enyl-4'-methoxychalcone,
3,5-dimethoxy-4-propyl-4'-methoxychalcone,
3,5-diethoxy-4-propyl-4'-methoxychalcone,
3,5-di-n-propoxy-4-propyl-4'-methoxychalcone,
3,5-diisopropoxy-4-propyl-4'-methoxychalcone,
3,5-di-n-butoxy-4-propyl-4'-methoxychalcone,
3,5-di-t-butoxy-4-propyl-4'-methoxychalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-methoxychalcone,

3,5-dimethoxy-2-methyl-4'-ethoxychalcone,
3,5-diethoxy-2-methyl-4'-ethoxychalcone,
3,5-di-n-propoxy-2-methyl-4'-ethoxychalcone,
3,5-diisopropoxy-2-methyl-4'-ethoxychalcone,
3,5-di-n-butoxy-2-methyl-4'-ethoxychalcone,
3,5-di-t-butoxy-2-methyl-4'-ethoxychalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-diethoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-ethoxychalcone,
3,5-dimethoxy-2-propyl-4'-ethoxychalcone,
3,5-diethoxy-2-propyl-4'-ethoxychalcone,
3,5-di-n-propoxy-2-propyl-4'-ethoxychalcone,
3,5-diisopropoxy-2-propyl-4'-ethoxychalcone,
3,5-di-n-butoxy-2-propyl-4'-ethoxychalcone,

3,5-di-t-butoxy-2-propyl-4'-ethoxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diethoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-propoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diisopropoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-butoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4'-ethoxychalcone,

3,5-dimethoxy-4-methyl-4'-ethoxychalcone,
3,5-diethoxy-4-methyl-4'-ethoxychalcone,
3,5-di-n-propoxy-4-methyl-4'-ethoxychalcone,
3,5-diisopropoxy-4-methyl-4'-ethoxychalcone,
3,5-di-n-butoxy-4-methyl-4'-ethoxychalcone,
3,5-di-t-butoxy-4-methyl-4'-ethoxychalcone,
3,5-dimethoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-diethoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-di-n-propoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-diisopropoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-di-n-butoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-di-t-butoxy-4-prop-2-enyl-4'-ethoxychalcone,
3,5-dimethoxy-4-propyl-4'-ethoxychalcone,
3,5-diethoxy-4-propyl-4'-ethoxychalcone,
3,5-di-n-propoxy-4-propyl-4'-ethoxychalcone,
3,5-diisopropoxy-4-propyl-4'-ethoxychalcone,
3,5-di-n-butoxy-4-propyl-4'-ethoxychalcone,
3,5-di-t-butoxy-4-propyl-4'-ethoxychalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-dimethoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,

3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-ethoxychalcone,

3,5-dimethoxy-2-methyl-4'-propoxychalcone,
3,5-diethoxy-2-methyl-4'-propoxychalcone,
3,5-di-n-propoxy-2-methyl-4'-propoxychalcone,
3,5-diisopropoxy-2-methyl-4'-propoxychalcone,
3,5-di-n-butoxy-2-methyl-4'-propoxychalcone,
3,5-di-t-butoxy-2-methyl-4'-propoxychalcone,
3,5-dimethoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-diethoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-di-n-propoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-diisopropoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-di-n-butoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-di-t-butoxy-2-prop-2-enyl-4'-propoxychalcone,
3,5-dimethoxy-2-propyl-4'-propoxychalcone,
3,5-diethoxy-2-propyl-4'-propoxychalcone,
3,5-di-n-propoxy-2-propyl-4'-propoxychalcone,
3,5-diisopropoxy-2-propyl-4'-propoxychalcone,
3,5-di-n-butoxy-2-propyl-4'-propoxychalcone,
3,5-di-t-butoxy-2-propyl-4'-propoxychalcone,
3,5-dimethoxy-2-(1,1-dimethylethyl)-4'-propoxychalcone,
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3,5-diisopropoxy-4-propyl-4'-propoxychalcone,
3,5-di-n-butoxy-4-propyl-4'-propoxychalcone,
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3,5-di-t-butoxy-4'-hydroxy)chalcone,

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3,5-di-t-butoxy-2-methyl-4'-hydroxychalcone,
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3,5-di-t-butoxy-2-(1,1-dimethylethyl)-4' hydroxychalcone,

3,5-dimethoxy-4-methyl-4'-hydroxy)chalcone,
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3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-hydroxy)chalcone,
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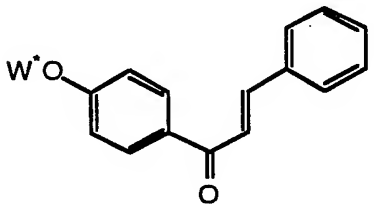
3,5-diethoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-di-n-propoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,
3,5-diisopropoxy-4-(1,1-dimethylethyl)-4'-hydroxy)chalcone,
3,5-di-n-butoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone and
3,5-di-t-butoxy-4-(1,1-dimethylethyl)-4'-hydroxychalcone,

46. Novel bis-aromatic α,β-unsaturated ketones of the general formula



wherein W* is R_H or a masking group as defined in claim 15 or 16, with the proviso that R_H is not methyl or ethyl.

47. A pharmaceutical composition which contains an aromatic compound, or a prodrug thereof, which aromatic compound contains an alkylating site and which aromatic compound is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, such as a compound as defined in any of claims 15-46 together with another antiparasitic, antimycotic, antibiotic or antibabesial drug or anticoccidial agent or another drug against fish parasites.

48. A pharmaceutical composition according to claim 47, wherein the other antiparasitic drug is an antileishmanial drug selected from a pentavalent antimony-sodium gluconate or allopurinol, or an antimalarial drug selected from chloroquine and derivatives thereof, quinine, proguanil, cycloguanil, mefloquine, pyrimethamine, and artemisinin.

49. A pharmaceutical composition according to claim 47, wherein the other antibabesial drug is selected from quinuronium sulphate, pentamidine isethionate, imidocarb or diminazene, or the other anticoccidial drug is selected from sulfonamides, amprocid and coccidiostatic agents selected from ionomycins, such as monensin and salinomycin, or the other drug used against fish parasites is selected

from benzimidazol and formaldehyde, or the additional antibiotic drug is an antituberculous drug selected from isoniazide, ethambutol, pyrazinamid, and rifampicin, or the additional antimycotic drug is selected from amphothericin B, muconarcidol, griseofluvin, and miconazol.

50. A composition which contains an aromatic compound (or a prodrug) as defined in any of claims 15-46 in combination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient suitable for administration to an animal, for the treatment or prophylaxis of a disease caused by a microorganism or a parasite as defined in claim 1.

51. A composition comprising a compound as defined in any of claims 35-46 in combination with an animal feed or drinking water for animals, or in combination with at least one pharmaceutical carrier or excipient.

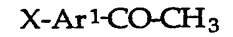
52. A composition according to any of claims 47-51 which is selected from a tablet, a suppository, and injection fluid.

53. A method for controlling transmission of parasitic diseases caused by parasites which have part of their life cycle in a vector in particular vector-born parasites among the parasites defined in claim 1, said method comprising applying an aromatic compounds, or a prodrug thereof, which aromatic compound contains an alkylating site and is capable of alkylating the thiol group in N-acetyl-L-cysteine at physiological pH, preferably a compound as defined in any of claims 15-46, to a locus which is a habitat of the vector so as to eradicate the parasites.

54. The method according to claim 53, wherein the application is performed by spraying a sprayable composition containing the aromatic compound.

55. A method for the preparation of a compound of the general formula I as defined in claim 15, said method comprising

a) for the preparation of a compound of the general formula I, in which both R are H, reacting a ketone of the general formula I'



I'

wherein X and Ar^1 are as defined in claim 15,
with an aldehyde of the general formula I''

I''

wherein Ar^2 and Y are as defined in claim 15, or

b) for the preparation of a compound of the general formula I in which W is -C≡C-, reacting an activated derivative of a carboxylic acid of the general formula II'

$$X\text{-}Ar^1\text{-}COOH \qquad II'$$

wherein X and Ar^1 are as defined in claim 15,

with an ethyne derivative of the general formula II''

$$H\text{-}C{\equiv}C\text{-}Ar^2\text{-}Y \qquad II''$$

wherein Ar^2 and Y are as defined in claim 15, or

c) for the preparation of a compound of the general formula I, in which W is -CR=CR-, wherein R is as defined in claim 15, dehydrating a β-hydroxyketone of the general formula E,

$$X\text{-}Ar^1\text{-}CO\text{-}CRH\text{-}C(OH)R\text{-}Ar^2\text{-}Y \qquad E$$

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 15, or

d) for the preparation of a compound of the general formula I, wherein W is -C≡C-, eliminating HLea from a ketone of the general formula E1,

$$X\text{-}Ar^1\text{-}CO\text{-}CH{=}C(Lea)\text{-}Ar^2\text{-}Y \qquad E1$$

wherein X, Y, Ar^1 and Ar^2 are as defined in claim 15, and Lea is a halide or another leaving group such as hydroxy, alkoxy, tosyloxy, or trifluoromethanesulfonoxy, or

e) for the preparation of a compound of the general formula I, wherein W is -CR=CH-, wherein R is as defined in claim 15, reacting an aldehyde or ketone of the general formula F

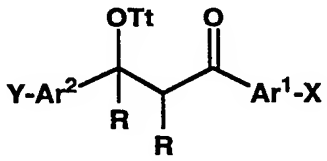
$$Y\text{-}Ar^2\text{-}CRO \qquad F$$

in which Y and Ar^2 are as defined in claim 15, with a phosphorus ylide (also called a phosphorane) of the general formula G,

T_3-P=CR-CO-Ar^1-X G

in which T is an aliphatic, alicyclic or aromatic group, and Ar^1, X and R are as defined in claim 15, or

f) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 15, eliminating HOTt from a ketone of the general formula K



K

wherein X, Y, Ar^1, Ar^2 and R are as defined in claim 15, and Tt is hydrogen, alkyl, tosyl, trifluoromethanesulfonyl or acyl, or

g) for the preparation of a compound of the general formula I, in which W is -CR=CR- in which R is as defined in claim 15, reacting a cinnamic acid of the general formula L

Y-Ar^2-CR=CR-COQ L

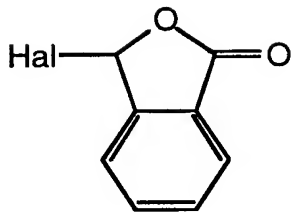
wherein Y, Ar^2 and R are as defined in claim 15, and Q is a hydroxy group, a carboxylate or a halogen atom, with an aromate of the general formula M

X-Ar^1 M

wherein X and Ar^1 are as defined in claim 15.

56. A method for preparing a compound of the general formula I as defined in claim 15, wherein Z is a group D or E as defined in claim 16, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ wherein A is as defined in claim 15, in particular -O- or -NH-, and Z is H, with the appropriate halide of the general fomula D-Hal or E-Hal

Hal-CR*R**-O-CO-R''' D-Hal



E-Hal

in which R*, R** and R''' are as defined in claim 16, and Hal is a halogen atom such as chlorine, bromine or iodine.

57. A method according to claim 56, wherein the halide D-Hal is iodomethyl pivaloylate.

58. A method for preparing a compound of the general formula I as defined in claim 15, wherein Z is a carboxylic acid residue (A) as defined in claim 16, comprising reacting the corresponding compound of the general formula I wherein X and/or Y is AZ, wherein A is as defined in claim 15, and Z is H, with a reactive derivative of the carboxylic acid HO-CO-R', wherein R' is as defined in claim 16, the reactive acid derivate being, in particular, selected from activated esters, anhydrides, and acid halides, such as the acid chloride.

59. A method for preparing a compound of the general formula I as defined in claim 15, wherein Z is a dimethylcarbamoyl group, comprising reacting the corresponding compound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 15, and Z is H, with an activated derivative of N,N-dimethyl-carbamic acid.

60. A method for preparing a compound of the general formula I as defined in claim 15, wherein Z is an alkoxyalkyl group (C) as defined in claim 16, comprising reacting the corresponding compound of the general formula I, in which X and/or Y is AZ, wherein A is as defined in claim 15, and Z is H, with an alkyl-α-haloalkyl ether.

61. A method for preparing a compound of the general formula I as defined in claim 15, wherein R is halogen comprising, reacting the corresponding compound of the general formula I in which W is -CH=CH- with bromine followed by dehydro-bromination of the formed dibromide using potassium acetate in methanol.

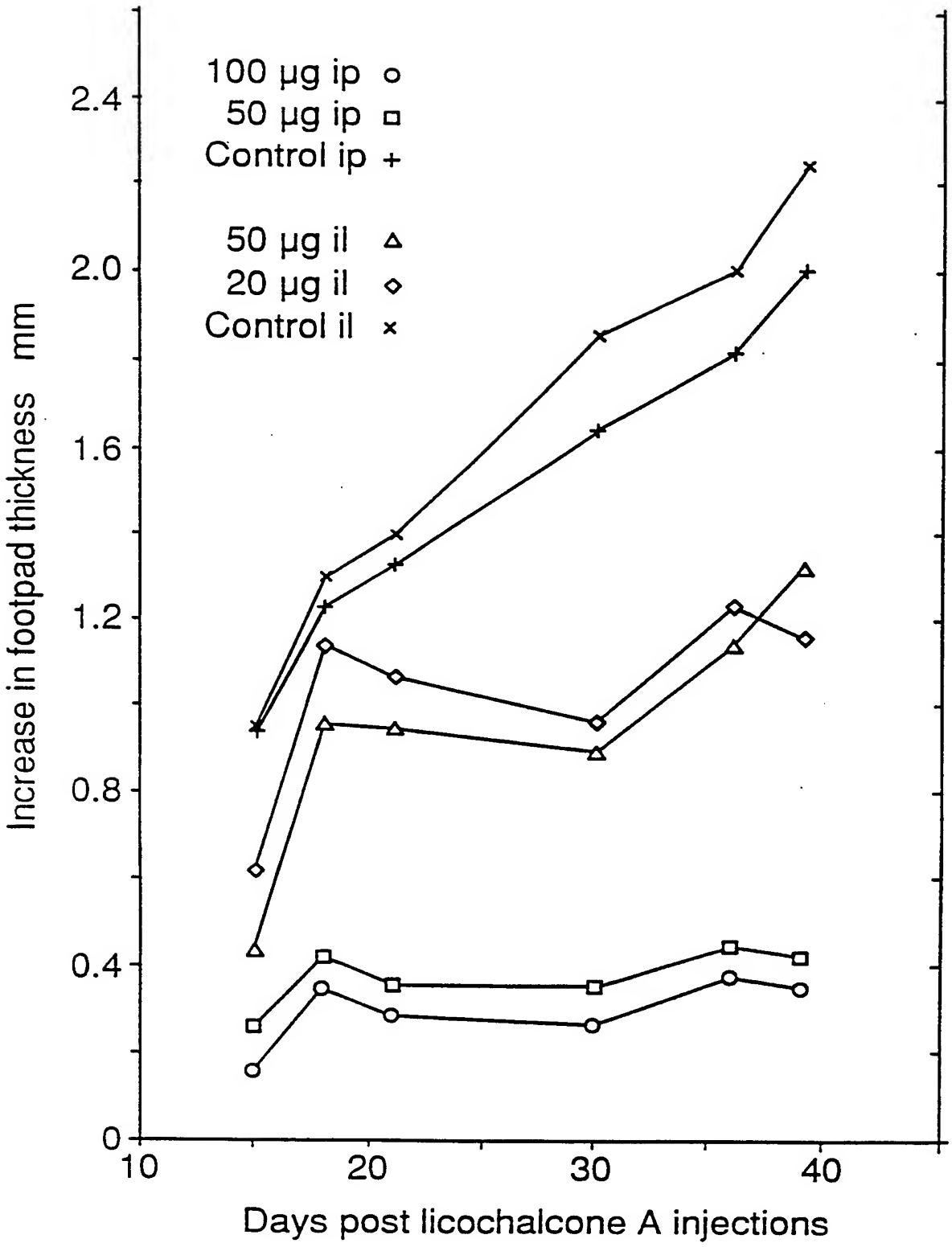

100 µg ip
50 µg ip
Control ip
50 µg il
20 µg il
Control il
Increase in footpad thickness mm
0
0.4
0.8
1.2
1.6
2.0
2.4
10
20
30
40
Days post licochalcone A injections

Fig. 1

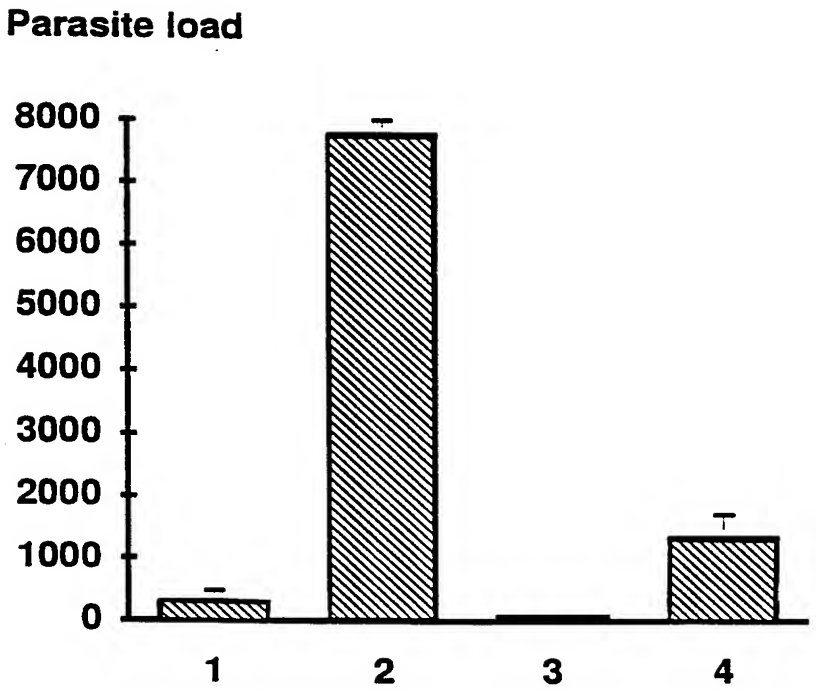


Fig. 2

INTERNATIONAL SEARCH REPORT

Inte. n. .pplication No
PCT/DK 94/00332

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C49/84 A61K31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TETRAHEDRON LETTERS., vol.23, no.15, 1982, OXFORD GB pages 1541 - 1544 V.S. KAMAT ET AL. 'A Versatile Total Synthesis of Xenognosin' see page 1542 ---	40,41
A	US,A,4 279 930 (C.M. HALL ET AL.) 21 July 1981 see the whole document ---	40,42,44
A	US,A,3 928 421 (K. KYOGOKU ET AL.) 23 December 1975 see the whole document ---	40,42,44
A	EP,A,0 292 576 (TSUMURA JUNTENDO,INC.) 30 November 1988 see claims -----	40,42,44

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
28 December 1994	4. 01. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bonnevalle, E

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ...pplication No
PCT/DK 94/00332

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4279930	21-07-81	NONE		
US-A-3928421	23-12-75	NONE		
EP-A-0292576	30-11-88	JP-A-	1042422	14-02-89
		JP-A-	63150241	22-06-88
		WO-A-	8804288	16-06-88
		US-A-	5106871	21-04-92
		US-A-	5234951	10-08-93